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# THE CHROMOSOME COMPLEMENT OF THE DEVELOPING EGGS PRODUCED BY DROSOPHILA PARTHENOGENETICA STALKER VIRGIN FEMALES

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FACULTATIVE parthenogenesis, leading to the production of adult progeny, has been reported in three species of the genus Drosophila, these being *D. parthenogenetica*, *D. polymorpha*, and *D. affinis* (STALKER 1951, 1952, 1953). Only one wholly parthenogenetic strain, this belonging to the species *D. mangabeirai*, has been described to date (CARSON, WHEELER and HEED 1957). Very infrequent parthenogenesis has been observed in *D. hydei*, *D. paramelanica* (STALKER, personal communication), and *D. robusta* (CARSON, personal communication).

The progeny produced by diploid virgin females of *D. parthenogenetica* were diploid and triploid females, diploid males, and 2n–3n mosaic females. Triploid virgin females produced the same kinds of progeny but in different proportions. The karyotypes of these offspring were determined by examining the chromosomes in the gonads and the cell size of the wings. All the diploid males were sterile, and analysis of the testes indicated that they were X0 rather than XY (STALKER 1953).

Genetic studies involving the use of the sex-linked, induced-mutant garnet, *g*, were made originally in order to determine the derivation of chromosome complements found in the progeny of virgin *D. parthenogenetica* females. STALKER (1953) concluded (largely from genetic data that parthenogenetic development in diploid females begins with two normal meiotic divisions; then two of the resulting haploid nuclei fuse to initiate diploid development, or three such nuclei fuse to initiate triploid development, or cleavage begins without previous nuclear fusion to initiate haploid development. Presumably the haploid cleavage would be responsible for many of the observed embryonic and early larval deaths.

Following studies of the progeny of XXY diploid virgins, STALKER (1953) concluded that the fusion of two haploid nuclei in unfertilized eggs may involve either terminal nuclei which are produced by the same secondary oocyte, or central nuclei which are produced by different secondary oocytes.

## THE PROBLEM

Unfertilized *D. parthenogenetica* eggs, ranging in age from one half to five hours after oviposition, were studied cytologically in an attempt to determine

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chromosomal behavior in the eggs and early embryos. There were two main objectives in this project.

The first objective was to confirm the occurrence of meiosis in a number of eggs and to see if fusion of certain of the resulting nuclei ever takes place.

The second objective was to determine the chromosome numbers in cleavage nuclei. Some embryos die before hatching. It was decided to determine whether these embryos were haploid due to nonfusion of meiotic nuclei or whether some other chromosome complement was involved.

## MATERIALS AND METHODS

The unisexual and bisexual strains of *Drosophila parthenogenetica* were derived from a single strain originally collected at Atlixco, Mexico, which was later established and supplied to STALKER by the University of Texas laboratory. STALKER raised the strain unisexually for 55 generations.

At the end of this time, females from the unisexual strain were mated to males from the original bisexual strain, and bisexual reproduction was carried out for several generations. A new unisexual strain was then extracted. This procedure was followed three times. Then a unisexual and a closely related bisexual strain were established. Both strains have been maintained since that time (spring 1956).

All flies were kept at 25°C and were reared in bottles on a standard cornmeal-Karo-agar-tegosept food medium to which a drop of yeast had been added.

Measurements of the rate of parthenogenesis were made for single virgin females. For this purpose each fly was kept in a separate vial and was transferred to fresh food each day. Each vial was examined under a dissecting microscope. A count was made of the number of eggs laid by the female. Counts were made on the second through the fifth days, after the female had been removed, to determine the numbers of dead embryos and larvae, the latter being removed as they were discovered. Eggs which had turned dark brown were considered to be evidence for dead embryos; STALKER (1953) found that such eggs contain larval tissues.

Studies were made of eggs of the unisexual strain during the first five hours of development and of eggs of the bisexual strain during the first three hours of development. Presumably the females of the unisexual strain were diploid. Although there is a remote possibility that some females were triploid, this is unlikely; the flies were at least two weeks old when they were studied and it is extremely difficult to keep triploid females alive for that period of time without special care.

The adults were etherized and placed in vials containing fresh food for at least 24 hours in advance of the time their eggs were to be collected; this was deemed necessary because flies often tend to hold back their eggs for several hours after they have been etherized, and it was desirable to know the age of the eggs which were to be studied. Egg-collecting chambers were prepared by inserting trape-

zoidal pieces of cardboard into standard vials. The paper had been covered with a thin layer of the food medium. Approximately ten flies were put into each chamber by attracting them to a bright light; shaking was thereby avoided so that the females would oviposit normally. The flies were removed from the vials one and one half to four hours later. Under a dissecting microscope the eggs were collected from the food by using a short dissecting needle with a spatula-shaped tip; the eggs were transferred to spot plate depressions containing several drops of distilled water. A number of eggs were "dechorionated" by adding an amount of sodium hypochlorite (in the form of commercial Chlorox) equal to the amount of water already present in the depressions. The mixture was stirred well, and the eggs were allowed to remain in the solution until the chorion was obviously loosened on each one; this usually took less than 30 seconds. The solution was then diluted by filling the depression completely with distilled water. The diluted solution was drawn off with a small-bore pipette. The eggs were then rinsed with distilled water in the depressions and fixed with a Carnoy fixing solution composed of equal amounts of chloroform, glacial acetic acid and 95 percent alcohol. Each egg was pricked with a very sharp dissecting needle; no care was taken to prick the egg at any particular place since it was desirable to study various areas in an intact state.

The eggs were stained by the standard Feulgen technique (DE TOMASI 1936; FAHMY 1950) and examined whole. The eggs were often kept in 70 percent alcohol for periods up to a week. Before the next step was taken, cocoons of ant pupae (COOPER and RIS 1943) were immersed lengthwise in 70 percent alcohol. Twenty to 40 eggs were put inside each cocoon which was then closed by a thread loop. Then the filled cocoons were transferred through the solutions to 95 percent alcohol. The cocoons were then broken open, the eggs were spread over the desired area, all debris was removed, and the eggs were mounted in diaphane.

Examinations of the eggs were made under oil immersion using Kohler illumination.

### *Description of normal development in fertilized eggs*

Stages occurring during the first one half to three hours of development after oviposition were studied in eggs laid by females of the bisexual strain. A total of 101 eggs was studied and all but three contained chromosomal material. The stages observed in the eggs that were definitely fertilized ranged from those in which the male and female pronuclei were approaching each other to those in which early organ formation was occurring. The majority of embryos were in the blastoderm phase.

In normal development the meiotic divisions occurred in a region approximately one third of the egg's length from the micropyle. The three polar nuclei migrated to the egg surface and remained in the anterior half of the egg. They could at first be distinguished from each other, but as cleavage proceeded, they formed a common mass of chromosomal material. These nuclei persisted at least

until Cleavage IV had been completed and did not appear to be degenerating at that time. In one case a polar body nucleus divided, but this did not appear to be a common occurrence.

The fusion of the two pronuclei and the first four cleavages took place deep within the egg cytoplasm. No eggs were observed showing stages between Cleavage IV and the early blastoderm. In the blastoderm stage the nuclei were all the same size and were also synchronous; this condition persisted until the very late blastoderm stage was reached at which time some nuclear size differences could be observed in the various regions of the embryo. During organ formation nuclear sizes became even more variable, and the nuclei were no longer completely synchronous.

The process of development in fertilized eggs was one of consistent regularity.

*Description of parthenogenetic development*

Stages occurring during the first one half to five hours of development after oviposition were studied in eggs laid by virgin females of the unisexual strain. Series I eggs were one half to two hours of age, and those of Series II were one to five hours old.

The premeiotic nucleus and the meiotic division nuclei were found always to be in a region about one third of the egg's length from the micropyle. In Series I and II, respectively, 29.1 percent and 83.8 percent of the eggs were without chromosomal material, 4.4 percent and 2.6 percent had degenerate premeiotic nuclei, and 4.9 percent and 0.2 percent had some chromosomal material but it was too degenerate to be classified according to the particular nuclear size or sizes represented (see Table 1). In the majority of Series I eggs, meiosis had not occurred, and in many Series II eggs this condition also seemed to be true. However, once meiosis had been completed, the resulting four haploid nuclei seemed to follow no consistent pattern. As indicated in Table 3, their positions varied considerably. In the majority of the eggs observed in the stages between ootid and first cleavage, the healthier appearing nuclei and most of the dividing nuclei were deep in the egg cytoplasm rather than on the surface. Peripheral nuclei were often degenerate and rarely showed normal cleavage divisions. Until Cleavage III had been completed no nuclear material was observed in the posterior quarter of the egg. Many of the eggs which had undergone meiosis and had started cleavage were obviously dying at the various stages of development. In general, the early cleavage nuclei followed no consistent developmental pattern.

Most of the eggs listed in Table 6 were in the blastoderm stage of development. Several were in a late cleavage stage, and two listed under the diploid or triploid category were in the process of early organ formation. Some of the haploid embryos were healthy looking and had no apparent degenerate nuclei. However, in the majority of embryos in which haploid nuclei were found, haploid cleavage did not seem to be proceeding normally; often chromosomal bridges were observed between the separating groups of daughter chromosomes in anaphase, and several times uneven divisions were seen. Some of the haploid-diploid or

TABLE 1

*Distribution of the types of chromosome complements found in the eggs and embryos of diploid virgin females of* Drosophila parthenogenetica

| Observed | Percent occurrence | |
|---|---|---|
| | Series I ½ to 2 hours | Series II 1 to 5 hours |
| No chromosomal material | 29.1 | 83.8 |
| Healthy premeiotic nucleus | 39.9 | 5.9 |
| Degenerate premeiotic nucleus | 4.4 | 2.6 |
| Too degenerate to classify | 4.9 | 0.2 |
| M I to ootid | 1.8 | 0.1 |
| Ootid to Cleavage I | 9.6 | 0.1 |
| Cleavage I to Cleavage II | 7.3 | 0.8 |
| Cleavage II to Cleavage III | 1.1 | 0.5 |
| Cleavage III | 0.2 | 0.0 |
| Possible early cleavage of haploid-diploid mosaics | 1.1 | 0.1 |
| Developing embryos | | |
| Haploid | 0.2 | 0.9 |
| Haploid-diploid or haploid-triploid mosaics | 0.5 | 2.1 |
| Haploid-diploid-triploid mosaics | 0.2 | 0.4 |
| Diploid or triploid | 0.0 | 2.2 |
| Diploid-triploid mosaics (or possibly diploid or triploid) | 0.0 | 0.1 |
| Total percent | 100.3 | 99.8 |
| Total number of eggs | 659 | 1586 |

haploid-triploid embryos consisted primarily of haploid nuclei which were not dividing normally.

The healthy eggs, which had reached the late blastoderm stage, were either diploid, triploid, or diploid-triploid mosaics with the exception of one haploid embryo observed in late blastoderm in which all nuclei appeared normal. In this case there had been at least one, and possibly two, additional cleavages beyond the point at which gastrulation normally occurs in the diploid so that the embryo consisted of many more nuclei than are normally found in a blastoderm stage. One embryo in the process of organ formation was definitely triploid; it consisted of fewer and larger nuclei than would be found in a diploid embryo in the same phase of development. Conclusions regarding the special haploid and triploid embryos were reached after comparing them with corresponding stages in fertilized eggs.

The impression reached after studying parthenogenetic development in the early stages was that there was no regular pathway of development which the eggs followed after oviposition. This is in sharp contrast to the situation in the fertilized eggs.

## RESULTS AND DISCUSSION

### *Preliminary considerations*

The eggs stained for Series I were not laid by the same females as those from

which Series II eggs were obtained. It cannot be assumed that the two groups are completely comparable; thus extensive correlations between them cannot be made. In Series I, at least 20.2 percent of the eggs had gone beyond the M I stage, while in Series II this was true for only 7.2 percent (see Table 1). This would seem to indicate that many of the eggs which complete meiosis die before the embryo has reached the stage of organ formation. This opinion is substantiated by the observation of numerous embryos dying in the various stages of development. Many eggs die without starting meiosis; in Series I, 4.4 percent of the eggs had a degenerate premeiotic nucleus as did 2.6 percent of the eggs in Series II (see Table 1). There is an indication that some eggs remain in an arrested stage and do not complete meiosis for at least 24 hours after they have been laid; evidence for this is found in the fact that larvae hatch over a 72-hour period from eggs which could not have varied in age by more than 24 hours.

### *Meiosis and cleavage*

*M I to ootid*: (see Table 2) Meiosis in the unfertilized eggs always occurred about one third of the egg's length from the micropyle, but the meiotic spindles seemed to have no consistent orientation, and the four resulting haploid nuclei did not appear to be arranged in any regular pattern.

*Ootid to Cleavage I*: (see Table 3) In both the "n n n" and "n 2n" groups, one haploid nucleus seemed to be missing. Presumably, it had degenerated before the designated eggs were fixed. The other groups were complete with regard to the amount of chromosomal material expected.

The "n n 2n" class included one egg in which both a haploid and a diploid nucleus were located deep in the egg, and in which the diploid nucleus was dividing; this would indicate that nuclei of different ploidy do not necessarily divide synchronously even when in a deep position.

*Cleavage I*: (see Table 4) Meiosis results in the production of four haploid

TABLE 2

*Analysis of the chromosomal material and the positions of the nuclei prior to the ootid stage in eggs of diploid virgin females of* Drosophila parthenogenetica

| Chromosomal material | Nuclear positions in egg cytoplasm | | Number of eggs observed | |
|---|---|---|---|---|
| | Surface | Deep | Series I ½ to 2 hours | Series II 1 to 5 hours |
| No chromosomal material | .... | .... | 192 | 1329 |
| Healthy premeiotic nucleus | .... | .... | 263 | 94 |
| Degenerate premeiotic nucleus | .... | .... | 29 | 42 |
| Too degenerate to classify | .... | .... | 32 | 4 |
| M I division | 1 nucleus | .... | 3 | 1 |
| Between M I and M II | 2 nuclei | .... | 3 | 1 |
| | .... | 2 nuclei | 5 | 0 |
| | 1 nucleus | 1 nucleus | 1 | 0 |

TABLE 3

*Analysis of the chromosome complements and the positions of the nuclei in the ootid to the first cleavage stage in eggs of diploid virgin females of* Drosophila parthenogenetica

| Chromosome complement | Nuclear positions in egg cytoplasm | | Number of eggs observed | |
|---|---|---|---|---|
| | Surface | Deep | Series I ½ to 2 hours | Series II 1 to 5 hours |
| M II division | 2 nuclei | .... | 3* | 0 |
| | .... | 2 nuclei | 2† | 0 |
| n n n n | n n n n | . . . | 5 | 0 |
| | n n | n n | 6 | 0 |
| | n | n n n | 3‡ | 0 |
| | .... | n n n n | 1 | 0 |
| n n n | n n n | ... | 1 | 0 |
| | .... | n n n | 0 | 2 |
| n n 2n | n n | 2n | 6 | 0 |
| | n | n 2n | 4§ | 0 |
| | n n 2n | . . . | 7 | 0 |
| n 2n | n 2n | .. | 2 | 0 |
| | n | 2n | 1 | 0 |
| n 3n | n 3n | ... | 9 | 0 |
| | n | 3n | 10 | 0 |
| | 3n | n | 3‖ | 0 |

* In two of these M II division eggs, the nuclei were abortive.
† In one of these M II division eggs, the nuclei were abortive.
‡ In one of these eggs containing four haploid nuclei, the nuclei were dividing.
§ In one of these eggs containing two haploid nuclei and one diploid nucleus, the diploid nucleus was dividing.
‖ In two of these eggs containing a haploid nucleus and a triploid nucleus, the haploid nucleus was dividing.

nuclei. If two of these fuse to produce a diploid nucleus, two haploid nuclei should remain. After the first division of the diploid nucleus, there should be two diploid and two haploid nuclei in the egg, unless one or both of the haploid nuclei also divided. In the "n 2n 2n" category, a haploid nucleus seemed to have degenerated already, leaving only one instead of the expected two or more.

In some classifications no haploid nuclei were present, indicating that all had degenerated if it is assumed that meiosis and fusion of two haploid nuclei had occurred. There is no direct way of proving that a two-by-two fusion of the four meiotic products did not occur in an egg showing only two diploid nuclei. Evidence for this cannot be expected from studies of progeny, since STALKER (1953) found no garnet mosaics among the 1565 diploid female progeny of the heterozygous $+/g$ virgin females.

In the "n n 2n 2n" group, one case was noted in which two haploid nuclei were dividing on the surface of the cytoplasm, but neither of two diploid nuclei on the surface was dividing; this indicates apparent asynchrony between nuclei of different sizes.

## TABLE 4

*Analysis of the chromosome complements and the positions of the nuclei from the first through the third cleavage stage in embryos of diploid virgin females of* Drosophila parthenogenetica

| Chromosome complement | Nuclear positions in embryonic cytoplasm | | Number of cases observed | |
|---|---|---|---|---|
| | Surface | Deep | Series I ½ to 2 hours | Series II 1 to 5 hours |
| | | **Cleavage I** | | |
| n n 2n 2n | n n | 2n 2n | 11 | 0 |
| | n n 2n | 2n | 1 | 1 |
| | 2n | n n 2n | 1 | 0 |
| | n 2n | n 2n | 1 | 0 |
| | n n 2n 2n | .... | 5* | 4 |
| n 2n 2n | .... | n 2n 2n | 1 | 0 |
| 2n 2n and chromosomal fragments | 2n 2n and fragments | .... | 2 | 0 |
| | fragments | 2n 2n | 0 | 1 |
| | 2n 2n | fragments | 1 | 0 |
| n n n n 2n 2n | n n n n | 2n 2n | 1 | 0 |
| 2n 2n | 2n 2n | .... | 5 | 1 |
| | 2n | 2n | 2 | 0 |
| | .... | 2n 2n | 3 | 3 |
| n n 3n 3n | n n 3n 3n | .... | 2† | 0 |
| | n n | 3n 3n | 1 | 0 |
| n 3n 3n | n 3n | 3n | 1 | 0 |
| | n | 3n 3n | 1 | 0 |
| 3n 3n | 3n 3n | .... | 1 | 1 |
| | .... | 3n 3n | 1 | 1 |
| Degenerate, either 2n 2n or 3n 3n | 2 nuclei | .... | 3 | 0 |
| | 1 nucleus | 1 nucleus | 1 | 0 |
| | .... | 2 nuclei | 1 | 1 |
| 2n, chromosomal material enough for n n n n | All nuclei | .... | 1 | 0 |
| 2n 2n 4n | 4n | 2n 2n | 1 | 0 |
| | | **Cleavage II** | | |
| 2n 2n 2n | .... | 2n 2n 2n | 3‡ | 2 |
| n n 2n 2n 2n | n n | 2n 2n 2n | 0 | 1§ |
| n n 2n 2n 2n 2n | .... | n n 2n 2n 2n 2n | 0 | 1 |
| | n n 2n 2n 2n 2n | .... | 0 | 1 |
| 2n 2n 2n 2n | .... | 2n 2n 2n 2n | 1 | 2 |
| | 2n 2n 2n 2n | .... | 1 | 0 |
| | 2n | 2n 2n 2n | 1 | 0 |
| n 2n 2n 2n 2n | 2n 2n 2n | n 2n | 1 | 0 |
| | | **Cleavage III** | | |
| 2n 2n 2n 2n | 2n 2n 2n 2n | .... | 1‖ | 0 |

* In one of these embryos having two haploid and two diploid nuclei, the haploid nuclei were dividing.
† In one of these embryos having two haploid and two triploid nuclei, a haploid nucleus was dividing.
‡ In one of these embryos having three diploid nuclei, one of the nuclei was dividing.
§ In one embryo with two haploid and three diploid nuclei, one of the diploid nuclei was dividing.
‖ In this embryo with four diploid nuclei, one of the nuclei was dividing.

In the "n n 3n 3n" class, an egg was observed in which only one haploid nucleus was dividing; this would indicate a possible asynchrony between nuclei of the same size.

*Cleavage II*: (see Table 4) In the "n n 2n 2n 2n" and "2n 2n 2n" groups, instances were noted in which the second cleavage was occurring asynchronously. One of two diploid nuclei produced by Cleavage I had cleaved prior to the division of the second, unless the origin of the third diploid nucleus can be attributed to the fusion of the two haploid cleavage nuclei.

The "n 2n 2n 2n 2n" group is another example of a case in which one of the two haploid nuclei remaining after fusion had presumably disappeared.

*Possibly early cleavage of haploid-diploid mosaics*: (see Table 5) An attempt will not be made to explain the various nuclear combinations observed in this category. There was sufficient indication in each case that haploid-diploid development might have occurred if the embryo had continued to live.

*Developing embryos*: The embryos listed in Table 6 were discussed in the

TABLE 5

*Analysis of the chromosome complements and the positions of the nuclei in possible early cleavage of haploid-diploid mosaic embryos of diploid virgin females of* Drosophila parthenogenetica

| Chromosome complement | Nuclear positions in embryonic cytoplasm | | Number of cases observed | |
|---|---|---|---|---|
| | Surface | Deep | Series I ½ to 2 hours | Series II 1 to 5 hours |
| n n n n n 2n 2n | n n | n n n 2n 2n | 1* | 0 |
| n n material,† n 2n | n n material | n 2n | 1‡ | 0 |
| n n n 2n | . . . | n n n 2n | 1 | 0 |
| | n | n n 2n | 1§ | 0 |
| n n n n n n n n 2n 2n | . . . . | n n n n n n n n 2n 2n | 1 | 0 |
| n n n n 2n 2n | n | n n n 2n 2n | 0 | 1 |
| n n n n n 2n, chromosomal fragments (part of egg missing) | n n n n and fragments | n 2n | 0 | 1 |
| n n n n n n 2n 2n 2n 2n 3n | 3n | n n n n n n 2n 2n 2n 2n | 1 | 0 |
| n n n n n n n 2n | . . . . | n n n n n n n 2n | 1 | 0 |

* In the embryo containing five haploid and two diploid nuclei, the three haploid nuclei located deep in the embryonic cytoplasm were dividing.

† In this embryo there were a healthy haploid nucleus and a healthy diploid nucleus. There were also two very degenerate haploid nuclei on the surface of the cytoplasm.

‡ In this embryo containing two degenerate haploid nuclei on the surface and a haploid and a diploid nucleus deep in the cytoplasm, the two deep nuclei were dividing.

§ In this embryo containing three haploid nuclei and one diploid nucleus, the two deep haploid nuclei were dividing.

TABLE 6

*Chromosome complements of developing embryos of diploid virgin females of* Drosophila parthenogenetica

| Type of development | Number observed | |
|---|---|---|
| | Series I ½ to 2 hours | Series II 1 to 5 hours |
| Haploid | 1 | 14 |
| Haploid-diploid or haploid-triploid mosaics | 3 | 34 |
| Haploid-diploid-triploid mosaics | 1 | 6 |
| Diploid or triploid | 0 | 35 |
| Diploid-triploid mosaics (possibly diploid or triploid) | 0 | 2 |

preliminary description of parthenogenetic development. It should be added that a number of these embryos were dying at the time of study. It is probable that some of these embryos would have died before they had advanced enough to have become discolored, thereby being recognized as "brown eggs."

As shown in Table 7, an average 12.3 percent of the eggs produced by the four

TABLE 7

*Rates of parthenogenesis for individual diploid virgin females of* Drosophila parthenogenetica *over a ten-day period*

| Female | Total eggs | | Percent brown eggs | Percent larvae |
|---|---|---|---|---|
| A | 639 | | 11.1 | 2.9 |
| B | 505 | | 9.5 | 2.8 |
| C | 773 | | 12.8 | 1.9 |
| D | 616 | | 7.0 | 1.3 |
| Total | 2,533 | Average | 10.1 | 2.2 |

females under consideration showed any detectable development. It is impossible to estimate how many of the eggs and embryos listed in Series I would have eventually developed to the brown egg or larval stage. In Series II it can be stated definitely that those eggs lacking chromosomal material, those having a degenerate premeiotic nucleus, and those containing chromosomal material too degenerate to classify would not have developed that far; these totaled 86.6 percent of all cases (see Table 1). It is probable that many of the remaining 13.4 percent would have failed to reach the brown egg or larval stage. The 3.5 percent, which were either partly or wholly haploid, and many of those in stages from Cleavage I through Cleavage III (1.3 percent), which showed some haploid development, would probably not have resulted in the production of larvae. It is impossible to determine what would have been the fate of the eggs containing premeiotic nuclei (5.9 percent); further development might have occurred in some instances. It will be noted that the proportion (2.3 percent) of cases considered to be diploid, triploid, or diploid-triploid mosaics is approximately the same as the proportion of hatching larvae (2.2 percent) (see Table 7).

Examples of nuclear size distribution in certain blastoderm embryos are listed in Table 5 and shown in Figure 1. It is difficult to determine the origin of the

first nucleus or nuclei of any one type in a particular embryo. It is not known whether each size of nucleus has a tendency to divide at its own characteristic rate or whether the haploid, diploid, and triploid nuclei usually divide with the same rapidity. Observations do not show whether or not one size tends to dominate or crowd out nuclei of other sizes in a particular region. In one blastoderm embryo, which is not illustrated in Figure 1, the majority of nuclei were diploid; the few haploid nuclei were just under the vitelline membrane and seemed to have been pushed out of the area in which the healthy peripheral nuclei of a blastoderm are found.

*Figure 1*: Embryo A consists of both haploid and diploid nuclei, each size occupying its own exclusive territory. Presumably the diploid nuclei were derived from an original diploid nucleus formed by the fusion of two meiotic products, and the haploid nuclei were the result of repeated divisions of one or both of the remaining meiotic haploid nuclei.

Embryo B consists of haploid nuclei with a somewhat different distribution than was found in Embryo A. The nuclear origins could have been the same as indicated for Embryo A, but the migration of the products of the first few cleavages seems to have been somewhat different.

Embryo C shows very unequal areas occupied by haploid and diploid nuclei. It seems likely that the development was originally haploid and that the first diploid nucleus or nuclei were the result of haploid fusion after the embryo had developed for a period of time. This type of fusion of haploid cleavage nuclei seemed characteristic of a number of embryos examined. This appears to have

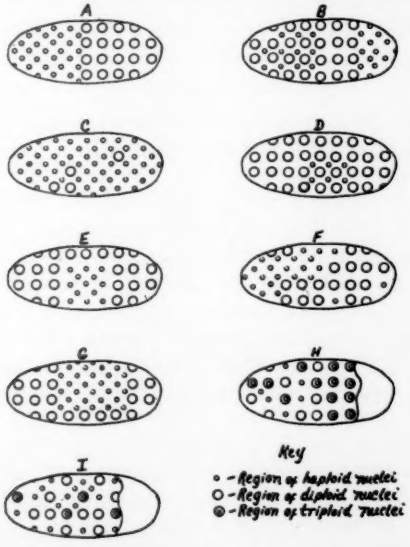


FIGURE 1.—Diagrams of representative mosaic embryos to show the variable distribution of haploid, diploid, and triploid nuclei.

occurred in the embryo consisting of seven haploid nuclei and one diploid nucleus, all located deep within the cytoplasm (see Table 5).

In haploid cleavage, daughter nuclei have been observed showing difficulty in separating during mitotic division. Chromosomal bridges between the nuclei at anaphase have been observed frequently. Possibly this phenomenon could result in incomplete separation and, thereby, in a diploid nucleus. A diploid nucleus might also be the result of fusion of two completely separate haploid cleavage nuclei.

Embryo D is the most difficult to explain. Either very extensive haploid fusion must be assumed or diploid cleavage must have occurred much more rapidly than haploid cleavage.

Embryos E, F, and G could have originated in the same way as did Embryos A and B. It is realized that there is a possibility in Embryos A, B, E, F, and G that the diploid nuclei resulted from the fusion of haploid cleavage nuclei. This seems less likely than in Embryo C, however, because the areas occupied by diploid nuclei are not, in any case, very much smaller than those in which haploid nuclei are found.

Embryos H and I are very similar. Both were dying at the time of study and the whole mitotic process seems to have been abnormal. Either the embryos could have started out as haploid-triploid mosaics and the diploid nuclei arose later by the fusion of haploid cleavage nuclei, or they could have started as haploid-diploid mosaics, with the triploid nuclei arising from the fusion of haploid and diploid nuclei. The first alternative seems more likely in the case of H, which contained more triploid nuclei than it did haploid or diploid. The second seems a more likely explanation for the origin of Embryo I, since it contained more haploid and diploid than triploid nuclei.

### *General considerations*

The categories listed for Series I and II indicate that meiosis occurred usually, if not always, before cleavage took place. Once meiosis had occurred, however, the nuclei followed no regular pattern.

In fertilized eggs the fusion of the two pronuclei and the first few cleavage divisions occurred deep within the cytoplasm, and it was not until the blastoderm stage that healthy dividing nuclei were found on the surface. Some nuclear divisions occurred on the surface in the cleaving unfertilized eggs, but generally the healthier and more successfully cleaving nuclei were found deep in the cytoplasm. Haploid cleavage was often irregular when it occurred in the egg center.

It has been proposed that in *Habrobracon juglandis* eggs, a substance or "agent" in the egg cortex usually destroys three of the four haploid nuclei resulting from meiosis, thus leaving one nucleus to participate with the sperm pronucleus in normal development. In these eggs, when two nuclear products of meiosis remain deep in the cytoplasm, both participate in development, one fusing with the male pronucleus and the other developing as a haploid nucleus. When this is the case, haploid-diploid mosaic individuals are produced (VON BORSTEL 1957).

It would also seem that with *D. parthenogenetica* only the nuclei which are

deep in the egg cytoplasm at an early stage are capable of repeated normal divisions.

Haploid and diploid nuclei found in the same embryo were sometimes dividing synchronously but were often asynchronous. In the early developing embryos, prior to blastoderm, the nuclei cleaving on the surface were usually not synchronous with those cleaving in the center. Diploid nuclei in fertilized eggs were not found on the egg surface until the blastoderm stage. However, many cases were observed in the unfertilized eggs in which either diploid or triploid nuclei were deep in the cytoplasm as well as on the surface. Presumably, the embryos in which this occurred would not have developed into larvae; the normal developmental process would probably have been interrupted when some cleavage products migrated to the surface and degenerated.

No adult progeny of virgin *D. parthenogenetica* females have been classified as haploid-diploid mosaics. In some of the wings examined, however, there were a few cases in which patches of very small, possibly haploid cells, were found (STALKER, personal communication). Among these progeny also, several flies were found in which part of the abdominal exoskeleton was missing, and others were found which had either five or, rarely, seven legs. It is possible that the missing abnormal areas were the result of irregular haploid cleavage. The rare seven-legged flies are not easily explained.

The major points of the discussion may be summarized as follows:

Meiosis occurred in many, if not in all, of the unfertilized eggs which later went through cleavage divisions. Haploid cleavage of meiotic products was observed. As haploid cleavage progressed, it often became irregular; chromosomal bridges could be observed between separating daughter nuclei at anaphase, and the division between them was sometimes unequal. Haploid meiotic products sometimes fused in twos to produce diploid cleavage nuclei or in threes to produce triploid cleavage nuclei.

The types of embryos developing from unfertilized eggs were haploid, haploid-diploid, or haploid-triploid mosaics, diploid, triploid, and diploid-triploid mosaics. The entire developmental process in unfertilized eggs was often irregular and this was in sharp contrast to the consistent regularity observed in fertilized eggs.

## SUMMARY

1. Studies were made of the development occurring during the first five hours in eggs of a unisexual strain of *D. parthenogenetica* and during the first three hours in a bisexual strain. The eggs were dechorionated, stained by the Feulgen technique, and examined as whole mounts under oil immersion, using Kohler illumination.

2. Observations made of the chromosome complements of unfertilized eggs are presented. In the eggs and embryos with detectable chromosomal material, the stages represented ranged from those having only a premeiotic nucleus to those in the process of early organ formation.

3. A description of the early development of fertilized eggs is given. The normal cleavage divisions were diploid, and the embryos showed consistent regu-

larity in their development. Only three of the 101 embryos lacked chromosomal material.

4. In unfertilized eggs, ranging in age from one half to two hours after oviposition (Series I), 29.1 percent of the 659 were without chromosomal material; this was true for 83.8 percent of the 1586 which were fixed one to five hours after oviposition (Series II).

5. In the unfertilized eggs, some meiotic divisions were observed. In many, if not in all of the developing embryos, meiosis had occurred. Observations of early cleavage with the various nuclear combinations indicate that diploid cleavage nuclei are often formed by the fusion of two haploid meiotic products and that triploid cleavage nuclei result from the fusion of three haploid meiotic products.

6. The healthier nuclei in preblastoderm embryos developing from unfertilized eggs were generally located deep within the egg cytoplasm; those in the cortex were usually degenerate and were not cleaving normally.

7. Early embryos sometimes contained only haploid, only diploid, or only triploid cleavage nuclei. In others both haploid and diploid or haploid and triploid cleavage nuclei were found together. Haploid cleavage was sometimes abnormal with anaphasic chromosomal bridges and unequal divisions between daughter nuclei being observed.

8. In some blastoderm embryos, both diploid and triploid cleavage nuclei were found. The interpretation depends upon the original development of the embryos. If it was haploid-triploid, the diploid nuclei could have resulted from an original fusion of two haploid nuclei or from multiple fusions of haploid cleavage nuclei in pairs. If the original development was haploid-diploid, the triploid nuclei could have arisen from a single fusion of a haploid and a diploid nucleus or from multiple haploid-diploid fusions.

9. The present study confirms the hypothesis of STALKER regarding the chromosomal behavior in unfertilized eggs of this species.

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# GENETIC ANALYSIS OF THE MALE FERTILITY FACTORS ON THE Y CHROMOSOME OF DROSOPHILA MELANOGASTER[1]

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THE Y chromosome of Drosophila has been the subject of many genetic studies, but by and large its genetic properties and organization have remained a mystery. The intractibility of the Y to genetic study is caused by the lack of clear-cut genetic effects (mutants) associated with it and by its unsuitability for classical genetic study (e.g., lack of crossing over, frequent position effect). The Y is certainly much more than a pairing partner for the X; however, unusual genetic techniques are generally necessary to demonstrate many of its genetic properties.

The Y chromosome of *Drosophila melanogaster* is wholly heterochromatic (HEITZ 1933) and is very poor in classical gene content for its cytological length. From a cytogenetic investigation of the base of the X and the Y, MULLER and PAINTER (1932) concluded that these heterochromatic regions were genetically inert. This seems to be substantiated by the lack of any phenotypic effect associated with the presence or absence of a Y in the male and female fly (BRIDGES 1916), both with respect to viability and to sex-linked characters, although the normal-appearing X/0 males are completely sterile. STERN (1929) showed that each arm of the Y carries a complex of factors necessary for male fertility. The function of these fertility factors is still not well understood, but they are in some way related to sperm maturation and motility. Studies of spermatogenesis in X/0 males (SAFIR 1930; SHEN 1932) revealed that meiosis occurs regularly and that most stages of spermiogenesis appear normal. However, the sperm apparently degenerate before the completion of maturation since very few mature sperm are found in the vasa efferentia of X/0 males and these sperm are never motile.

In addition to the fertility genes, the Y carries a region that is homologous to part of the base of the X. STERN (1927, 1929) established that a wild type allele of bobbed ($bb^+$), a gene located in the proximal heterochromatin of the X, was represented on the Y. HEITZ (1934) found that a nucleolus-organizing region was present on both the X and the Y. The nucleolus organizer and probably $bb^+$ are on the short arm of the Y ($Y^S$) (see COOPER 1959 for discussion). Further evidence for homology between $Y^S$ and the base of the X comes from studies of crossing over between the X and the Y (STERN 1929; KAUFMANN 1933; PHILIP 1935; NEUHAUS 1936, 1937; LINDSLEY 1955a,b). These exchanges occur very infrequently and may be mitotic in origin (gonial). These studies indicate that $Y^S$ is

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the arm most frequently involved in spontaneous exchange (see LINDSLEY 1955b for discussion). Finally, NEUHAUS (1938, 1939) gave evidence for a fertility factor common on the base of the X and the long arm of the Y ($Y^L$), but the existence of this factor and its possible location are not definitely established.

From a cytological study of pairing in meiosis in males, COOPER (1949, 1952) concluded that pairing between the X and the Y occurs at specific sites or collochores. There are two of these collochores on $Y^S$ and perhaps two more on $Y^L$. Whether these collochores are regions of true homology is not certain, for in certain chromosomal rearrangements the base of the X seems to be able to pair with the fourth chromosome (SANDLER and NOVITSKI 1956) as can the Y (GRELL, 1959b; personal communication). LINDSLEY's analysis (1955b) of crossing over in XY compounds indicates that, if the collochores on the X and Y are regions of homology, these regions have no polarity and are equal to each other. The regular disjunction of the X and Y in normal males clearly indicates that these elements share a homologous region. Some of the detachment products produced by crossing over in the tandem metacentric and tandem acrocentric compound X chromosomes are lethal if no homolog is present in the meiotic nucleus. The presence of a Y greatly increases the frequency of single exchanges in the reversed acrocentric compound X (SANDLER 1954) but has no effect on crossing over in the reversed metacentric or on the autosomes. This effect is specific for the Y inasmuch as it is not duplicated by a free duplication for the base of the X.

Other genetic properties of the Y are even less clear-cut. The Y chromosome has a very strong modifying effect on V-type position effect (see LEWIS 1950 for review). The presence of an extra Y chromosome suppresses the variegation, making the phenotype more like the normal (GOWEN and GAY 1933, 1934; DUBININ and HEPTNER 1935; SCHULTZ 1936), whereas the absence of a Y enhances it (NOUJDIN 1936). The factors responsible have not been located but are probably present on both arms of the Y (NOUJDIN 1938). From work of BAKER and SPOFFORD (1958), it seems that discrete functional units may be responsible for the effect of the Y on variegation inasmuch as the ability of Y-chromosome fragments to suppress variegation is not related to their size.

It has been suggested that the Y chromosome carries a system of polygenes (BARRIGOZZI 1948, 1951; BARRIGOZZI and DI PASQUALE 1953; MATHER 1944; and others). These studies are based on minor differences between males and females in quantitative characters such as the number of sternopleural chaetae and the number of wing hairs. The evidence in favor of polygenes on the Y is by no means compelling; in fact in one case, where the comparison was made, there was no difference between the X/0 and X/Y males. COOPER (1945) pointed out the questionable nature of MATHER's (1944) results. NEUHAUS (1939) presented evidence for an effect of the Y on several metric characters, but again the data are only suggestive.

Other effects become evident when supernumerary Y's are present. Although the gross appearance of the flies and the sex balance are unaffected, flies with two Y's more than the normal complement have mottled eyes (COOPER 1956), and the percentage of flies with mottled eyes is related to the number of extra Y

arms. Males with three Y's are sterile (SCHULTZ in MORGAN, BRIDGES and SCHULTZ 1934; COOPER 1956), and four Y's are probably lethal (COOPER 1949). Female fertility is slightly reduced by one Y and strongly reduced by two (GRELL 1959a). The effect of Y hyperploidy on the fertility of the male and female is not dependent on the dose of the fertility factors but may depend on a factor or factors proximal to the fertility genes (COOPER 1956).

Work on the effect of a Y chromosome in the female on nucleic acid composition and content of certain cells has yielded new clues to a possible functional activity of the Y (see SCHULTZ 1956 for review). Females with a Y produce eggs that have a higher amount of ultraviolet-absorbing material (CASPERSSON and SCHULTZ 1938), which at first suggested that these eggs had an increased amount of nucleic acids. Later work demonstrated, however, that the total amount of ribonucleic acid (RNA) is essentially unaffected (CALLAN 1948; SCHULTZ 1956), but that its composition is altered. LEVENBOOK, TRAVAGLINI and SCHULTZ (1958) analyzed the base composition of the RNA in X/X and X/XY (the attached-XY chromosome) eggs and found that the X/XY eggs had significantly more adenine than X/X eggs. This altered RNA has a higher specific ultraviolet absorption than normal RNA, which accounts for the earlier results. Identification of the base composition of hydrolyzates of cold perchloric acid extracts of eggs of different genotypes reveals further differences. The purine content of X/XY eggs is more than double that of X/X eggs; principally owing to adenine-containing compounds, which are increased almost four times. The total amount of pyrimidine is about the same in the two genotypes; however, the X/XY eggs have only a trace of thymine and a large amount of cytosine, whereas the reverse is true for the X/X eggs (TRAVAGLINI, LEVENBOOK and SCHULTZ 1958). The XY chromosome is abnormal in its heterochromatic composition in that some of the heterochromatin from the proximal part of the X is present and it is likely that part of the Y heterochromatin is absent. Therefore, some of these studies were extended to include the effect of a normal Y. Comparisons were made between eggs from attached-X ($\widehat{XX}$) females with and without a Y. The same general picture was obtained except that the increase in thymine in the $\widehat{XX}$/Y eggs was only one sixth as great as the increase in the X/X eggs. The authors suggest that the effect of the Y is on the synthesis of nucleic acid precursors that accumulate in the unfertilized and to some extent in the fertilized X/XY egg. It is clear that disruption of the heterochromatin-euchromatin balance by addition of a Y chromosome affects nucleic acid metabolism but it is not yet clearly established that the Y normally has a significant role in the control of nucleic acid synthesis. An extra Y has no measurable effect on the amount of RNA in salivary gland cells (PATTERSON, LANG, DACKERMAN and SCHULTZ 1954) or in the adult fly (ALTORFER 1953). The Y does increase the amount of DNA in follicular and nurse cells (FREED and SCHULTZ 1956). This effect is independent of ploidy and is greater than can be accounted for by the DNA of the Y itself.

It is obvious from this brief survey that our present knowledge of the genetic properties of the Y chromosome are far from satisfactory. Of the known genetic

factors on the Y, the fertility genes provide the most promising approach to a further analysis of the Y chromosome. The first two questions to be answered about these factors are: how many genes are there in each complex and can they be linearly ordered? The results of these studies show that there are at least two fertility genes on $Y^S$ and at least five on $Y^L$. These genes can be linearly ordered on the chromosome.

### *Terminology*

An unambiguous symbolic representation for the various altered Y chromosomes described in this paper required a new terminology. A system for noting the constitution of XY compound chromosomes has already been presented by LINDSLEY and NOVITSKI (1959). STERN (1929) originally represented the fertility complexes by the symbol *K*, where $K_1$ is the fertility complex of $Y^L$, and $K_2$ that of $Y^S$. This system has serious limitations when the complexes are resolved into their components. The *K* can be retained and the $Y^L$ complex as a whole symbolized by *KL* and the $Y^S$ complex by *KS*. Lower case letters with a numeral added designate individual loci. The fertility genes of *KL* are arranged numerically starting at the centromere end of the complex, e.g., *kl-1*, *kl-2* . . . *kl n*, as are those of *KS*., *ks-1*, *ks-2* . . . *ks n*. If more loci are described in the future, the numbering system of the appropriate complex will be shifted so that the numerical order of the loci is maintained. In accord with the usual Drosophila notation, the normal allele is indicated by a "+" or specifically *kl-1*$^+$, *kl-2*$^+$, etc., and the mutant alleles are *kl-1*, *kl-2*, etc. A sterile Y is designated as $Y^{KL-}$ or $Y^{KS-}$, depending on the location. The complete notation of an XY compound chromosome, such as the attached-XY, is $Y^S$X·$Y^L$; *In(1)EN; KL y·KS y*$^+$ (LINDSLEY and NOVITSKI 1959). The raised dot indicates the position of the centromere. In this paper this particular compound chromosome will be referred to simply as the attached—XY.

The symbols used in this paper are as follows: achaete (*ac*); Bar (*B*); bobbed (*bb*); bobbed-deficiency (*bb*-Df); brown (*bw*); Inversion (1) EN (*In(1)EN*); Inversion (1) delta-49 (*In(1)*dl-49); Inversions (2L + 2R) Curly (*Ins(2L + 2R)Cy*); Inversion (3LR) Dichaete (*In(3LR) DcxF*); miniature (*m*); vermilion (*v*); scute (*sc*); scute$^8$ ($sc^8$); scute$^{81}$ ($sc^{81}$); shaven naked ($sv^n$); white (*w*); yellow (*y*); and Y chromosome long arm, closed ($Y^{cl}$).

### *Recovery of the sterile Y chromosomes*

Normally a Y chromosome with a mutation or deficiency in the fertility factor complex (a sterile Y) would be eliminated as soon as it arose owing to the inability of males receiving it to leave progeny. This difficulty can be circumvented by the use of the attached-XY chromosome, which carries attached to a single centromere all the sex-chromosome material necessary for male viability and fertility. Males carrying a normal X marked with yellow (*y*) and $sc^8$·Y [a Y carrying as a duplication the tip of the X including the marker *y*$^+$ (MULLER 1948)] were irradiated with 3000r of X-rays and mated to homozygous XY, *y B* females. If any of the $F_1$ sons receives a sterile Y, he will still be fertile owing to the normal

fertility genes on the attached-XY. The $F_1$ males were pair-mated to *In(1)*dl-49, *y w m*/XY, *y B* females. The X part of the XY is in inverted order, thus in the heterozygote with *In(1)*dl-49, the two chromosomes differ by two inversions and crossing over is almost completely absent. After the females were allowed to ovaposit for six days, the parents were discarded. When these cultures were 15 days old, the progeny were shaken into a fresh culture vial and subsequently were discarded after six days. This progeny should contain males of two types: XY, *y B*/Y* and *In(1)*dl-49, *y w m*/Y* where Y* is the irradiated Y. Absence of a paternally derived dl-49 X chromosome in the next generation indicates the presence of a damaged Y, $Y^{K-}$. In practice this was recognized by the absence of *y w* non-*B* females among the progeny. These crosses are summarized in Table 1. The presence of *w*, non-*B* females can easily be detected by examination through the side of the vial, and a large number of cultures can be examined quickly. Those cultures in which no *w*, non-*B* females were found were retained for further testing since they were presumed to carry a sterile Y. The *In(1)*dl-49, *y w m*/Y* males were isolated and tested for fertility, and the XY, *y B*/Y* males were crossed to attached-X females to propagate the stock.

A total of 1650 $sc^8$·Y chromosomes were screened for fertility, and 57 putative sterile Y's were recovered. In addition to these, there were six sterile Y's recovered in the $F_1$ on the basis of loss of the $y^+$ markers. Actually a large number of $y^+$ losses were found, but unless they carried both $bb^+$ and one or the other fertility complexes, they were presumed to be deleted X's or whole Y-chromosome losses and the cultures were discarded. The sterile Y's were retested by crossing the XY, *y B*/$Y^{K-}$ males to free X females and testing the $F_1$ males for fertility. In a number of the cultures, the marker $y^+$ did not segregate regularly from the XY chromosome. In these cases it was assumed that $y^+$ had been transferred to one of the autosomes, and these cultures were discarded. The possibility

TABLE 1

*The crosses used to detect and recover sterile Y chromosomes*

| | | | |
|---|---|---|---|
| P | XY, *y B*/XY, *y B* | × | *y*/$sc^8$·Y*, $y^+$ |
| $F_1$ | XY, *y B*/*In(1)*dl-49, *y w m* | × | XY, *y B*/$sc^8$·Y*, $y^+$ |
| $F_2$ | Females | | Males |
| | XY, *y B*/XY, *y* B | × | XY, *y B*/$sc^8$·Y*, $y^+$ |
| | XY, *y B*/*In(1)*dl-49, *y w m* | | *In(1)*dl-49, *y w m*/$sc^8$·Y*, $y^+$ |

| $F_2$† Female gametes | Male gametes | | |
|---|---|---|---|
| | XY, *y B* | *In(1)*dl-49, *y w m* (when $sc^8$·Y* is fertile) | *In(1)*dl-49, *y w m* (when $sc^8$·Y* is sterile) |
| XY, *y B* | *y*, narrow-*B* female | *y*, wide-*B* female | None |
| *In(1)*dl-49, *y w m* | *y*, wide-*B* female | *y w m*, non-*B* female | None |

* The males carrying this Y chromosome were irradiated with 3000r of X-rays. The irradiated Y is marked with an asterisk so that it may be followed through the crosses used.

† In the $F_2$ cross only the gametes giving rise to females are considered. Cultures that did not have *w*, non-*B* females were presumed to carry a sterile Y and were saved for retesting.

remains, however, that some of these cultures had Y chromosomes in which the pairing sites (collochores) had been deleted along with some of the fertility genes. In this event, deficiences for fertility genes closely linked to collochores would be selected against.

The sterile Y cultures that remained were tested to determine the affected arm. Attached-X females with the sterile Y's were crossed to X's with a single Y arm attached, $X{\cdot}Y^L$ or $X{\cdot}Y^S$. If the sterile Y is fertile with $X{\cdot}Y^S$ but not with $X{\cdot}Y^L$, then the sterility mutation is on $Y^S$; the converse is true for $Y^L$ mutations. These sterile Y cultures were tested for autosomal translocation using *Ins(2L + 2R)Cy* for chromosome II, *In(3LR)DcxF* for the third, and $sv^n$ for the fourth. All the translocations found were discarded lest the rearrangement cause a position effect and possibly yield results that would be difficult to interpret. All sterile Y's that survived these tests were presumed to be free of gross interchromosomal rearrangements and to carry mutations or deletions in the fertility regions. In this group there were 33 $Y^{KL-}$'s and 12 $Y^{KS-}$'s. When the $\gamma^+$ losses were added we had 37 $Y^{KL-}$'s and 13 $Y^{KS-}$'s. The complementation tests described in the next section were carried out with 30 of the $Y^{KL-}$'s and 11 of the $Y^{KS-}$'s. The Y's that were excluded included the $\gamma^+$ losses, and three $Y^{KL-}$'s that were lost before they could be tested, and one $Y^{KS}$ that spontaneously reverted to fertility. This latter reversion was probably not a reverse mutation, but rather the result of an exchange with XY and is discussed in a later section.

### *Complementation tests*

Females that were $\gamma\ v/\ \gamma\ v/$ Y:$bw^+$; *bw* (a normal Y carrying as an insertion a duplication for a small part of the second chromosome including the locus $bw^+$) were crossed to XY, $\gamma$ *B*/$Y^{K-}$, $\gamma^+$ males. The $\gamma v$/Y:$bw^+$/$Y^{K-}$, $\gamma^+$;*bw* males in the progeny were then crossed to $\overset{\frown}{XX}$, $\gamma$/$Y^{K-}$, $\gamma^+$;*bw* females. Among the progeny of this cross are two kinds of $\gamma^+v$; *bw* males in equal frequency, those with one $Y^{K-}$, $\gamma^+$ and those with two. If the $Y^{K-}$ from the female was different from the $Y^{K-}$ from the male, resulting $Y^{K-}/Y^{K-}$ males would be heterozygous for two different sterile Y's. Furthermore, this particular type of male could be made up in two ways depending on which parent contributed which $Y^{K-}$. Any two sterile Y's could be tested for complementation by testing the proper $X/Y^{K-}/Y^{K-}$ males for fertility. In this manner all possible combinations of $Y^{KL-}$ and $Y^{KS-}$ were made up and tested. Each of the possible combinations were made reciprocally so that each test was replicated. In all cases where the results of the replicate tests were discordant a retest was made. The very few discordant tests found are discussed later.

The actual complementation test consisted of placing 40-50 $\gamma\ v/Y^{K-}/Y^{K-}$; *bw* males in a bottle with 30 $\gamma\ v/\gamma\ v$;*bw* virgin females. The resulting cultures were classified as sterile or fertile on the basis of the presence or absence of larval tunnels on the food after seven days. The progeny of the fertile cultures were checked for evidence of nonvirginity of the test female and for possible inclusion of X/Y:$bw^+$/$Y^{K-}$ males among the test males. As pointed out in the preceding

paragraph, the $X/Y^{K-}/Y^{K-}$ males were phenotypically identical to their $X/Y^{K-}$ brothers; therefore 80–100 $y^+v;bw$ males were actually used in the test, ensuring the presence of an adequate number of the desired males. At a later time it was brought to my attention that males with two $sc^s$·Y, $y^+$ chromosomes could be distinguished by the presence of hairs in the second posterior cell of the wing. This phenotype was found to be reliable by progeny test of males with one or two normal $sc^s$·Y, $y^+$ chromosomes and was used to identify the proper males in all retests. Results of these tests and the few necessary retests are given in the following sections.

## *Tests with* KS

Results of the complementation tests with the $Y^{KS-}$'s are given in Table 2, where it can be seen that there are three types. Sterile Y's 2, 4, 6, 7, 8, 10, 11, 12, and 13 are all sterile *inter se*, which indicates that they share a deficiency or mutation for the same locus. Since all members of this group are fertile with Y5, Y5 must be mutant at a different locus; therefore at least two fertility loci must be present on $Y^s$. Sterile Y 14 is sterile with either group and hence must lack both loci. This latter chromosome is the only sterile Y in the whole series (including the $Y^{KL-}$'s) that is also deficient for $bb^+$. Since there are only two genes in *KS*, no conclusions about the organization of the genes in this region can be drawn.

The relative frequency of breakage in the four regions defined by the centromere, $bb^+$, the two genes of *KS*, and the end of the chromosome gives an idea of the spatial separation of the fertility genes and their relative positions on the chromosome. This argument is based on the assumption that all the sterile Y's have deficiencies with two breakpoints (although these may be very close to each other). Alternative hypotheses such as differential mutability of the genes and differential sensitivity of the regions to X-rays cannot be ruled out. The gene deficiency represented by the group of nine $Y^{KS-}$'s has been designated *ks-1* and

TABLE 2

*Results of complementation tests with the* $Y^{KS-}$*'s*

| Male | Number of $Y^{KS-}$ introduced through the Female | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 4 | 5 | 6 | 7 | 8 | 10 | 11 | 12 | 13 | 14 |
| 2 | X† | — | F | — | — | — | — | — | — | — | — |
| 4 | — | X | F | — | — | — | — | — | — | — | — |
| 5 | F | F | X | F | F | F | F | F | F | F | — |
| 6 | — | — | F | X | — | — | — | — | — | — | — |
| 7 | — | — | F | — | X | — | — | — | — | — | — |
| 8 | — | — | F | — | — | X | — | — | — | — | — |
| 10 | — | — | F | — | — | — | X | — | — | — | — |
| 11 | — | — | F | — | — | — | — | X | — | — | — |
| 12 | — | — | F | — | — | — | — | — | X | — | — |
| 13 | — | — | F | — | — | — | — | — | — | X | — |
| 14 | — | — | — | — | — | — | — | — | — | — | X |

† X, All homozygous combinations sterile; F, fertile combination; and —, sterile combination.

the other factor *ks-2*. Of a total of 22 breaks recovered, ten were between *ks-1* and *ks-2* whereas only two were distal to *ks-2* (assuming the most probable order of the genes to be centromere, $bb^+$, *ks-1*, *ks-2*). Nine breaks between *ks-1* and $bb^+$ were recovered but only one break proximal to $bb^+$ was found. This paucity of breaks proximal to *ks-1* and $bb^+$ may be caused by selection of $Y^{K-}$'s that disjoined normally from the XY since this region probably contains the collochores also. Thus these data, though only tentative, suggest that *ks-1* and *ks-2* are probably fairly well separated and that *ks-2* is near the end of $Y^s$.

### *Tests with* KL

The results of the complementation tests with the $Y^{KL-}$ chromosomes are given in Table 3. The chromosomes that gave identical results in the test crosses presumably have identical deficiencies and are grouped together in Table 3. The 30 sterile Y's fall into 12 unique groups. The first step in the analysis of this table is to define the genetic relation between these groups. We can assume that the group headed by 13 is deficient for a single locus that we can arbitrarily designate as position 1 and is normal or + for an as yet undetermined number of other loci. Then, since 37 complements all the members of the first group, 37 must be + at position 1 and deficient somewhere else, for instance position 2. Number 41 complements both the first two groups and hence is + for positions 1 and 2 but deficient somewhere else, say position 3. This procedure can be repeated for all groups. Results of this analysis are given in Table 4. The data can be completely

TABLE 3

*The results of complementation tests with* $Y^{KL-}$

| Male | Number of $Y^{KL-}$ introduced through the: Female | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 13<br>25<br>29<br>33 | 37 | 11<br>20<br>27<br>41 | 12<br>23 | 3<br>28<br>34 | 36 | 38 | 7<br>8<br>14<br>18<br>19<br>24<br>39 | 32 | 10 | 1<br>5<br>9<br>15 | 4 |
| 13, 25, 29, 33 | X† | F | F | F | F | F | F | F | — | — | F | — |
| 37 | F | X | F | — | F | F | F | F | — | F | — | — |
| 11, 20, 27, 41 | F | F | X | — | F | F | — | — | — | — | — | — |
| 12, 23 | F | — | — | X | F | F | — | — | — | — | — | — |
| 3, 28, 34 | F | F | F | F | X | — | F | — | F | F | — | — |
| 36 | F | F | F | F | — | X | — | — | F | F | — | — |
| 38 | F | F | — | — | F | — | X | — | — | — | — | — |
| 7, 8, 14, 18, 19, 24, 39 | F | F | — | — | — | — | — | X | — | — | — | — |
| 32 | — | — | — | — | F | F | — | — | X | — | — | — |
| 10 | — | F | — | — | F | F | — | — | — | X | — | — |
| 1, 5, 9, 15 | F | — | — | — | — | — | — | — | — | — | X | — |
| 4 | — | — | — | — | — | — | — | — | — | — | — | X |

† See footnote Table 2.

explained on the basis of five genes. Table 4 includes five additional sterile Y's that were not in the original complementation tests. The genotypes of these Y's were determined by testing against known deficiencies.

Eight of the 12 types of deficiencies are multiple gene deficiencies, several of them overlapping each other. All but one of these deficiencies are continuous, that is to say only adjacent genes are missing in any given deficiency. This observation is consistent with the hypothesis that the genes are linearly ordered on the chromosome. Number 10 is an apparent exception to this rule in that it seems to complement 37. The reason for this is not clear. The combination 10/37 does not restore full fertility to the male, but the combination consistently showed some complementation in three retests. It is not unreasonable that one multiple-break event should be recovered among 41 sterile Y's from an exposure of 3000r. Thus this case probably does not invalidate the rule. The organization of the fertility complex can be best pictured as a linear array of particulate units. In this respect this heterochromatic region is organized in a manner similar to the euchromatic regions of the chromosomes. The results of the analysis of *KL* are given in the form of a complementation map in Figure 1. It is obvious that there are fewer breaks between *kl-4* and *kl-5* and perhaps between *kl-3* and *kl-4* than between the other genes. This could mean that these genes are less widely separated spatially than are the others. However, again it is impossible to rule out differential radiation sensitivity.

In all the original experiments, the sterile Y's seemed to sterilize the male completely. But experiments undertaken for other purposes have revealed that some of the sterile Y's have a low level of fertility. The results of fertility tests with these very low fertility sterile Y's are given in Table 5. Two of these sterile Y's are multiple gene deficiencies. Although it may be imagined that loss of *kl-5*$^+$ or *kl-3*$^+$ alone might permit a low level of fertility, some other explanation is required for these multiple gene deficiencies. One hypothesis that might be

TABLE 4

*Genotypes of the* $Y^{KL-}$*'s*

| | Sterile Y number | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fertility factor no. | (30)† 13 25 29 33 | 37 | 11 20 27 41 | 12 23 | 3 28 34 | 36 | 38 | (102) (103) 7 8 14 18 19 24 39 | 32 | 10 | 1 5 9 15 | (101) (105) 4 |
| *kl-1* | − | + | + | + | + | + | + | + | − | − | + | − |
| *kl-2* | + | − | + | − | + | + | + | + | − | + | − | − |
| *kl-3* | + | + | − | − | + | + | − | − | − | − | − | − |
| *kl-4* | + | + | + | + | + | − | − | − | + | + | − | − |
| *kl-5* | + | + | + | + | − | − | + | − | + | + | − | − |

† Numbers in parentheses refer to additional $Y^{KL-}$'s not included in the experiments comprising Table 3.

TABLE 5

*Fertility of the partially fertile $Y^{KL-}$ chromosomes*

| $Y^{KL-}$ number | No. males tested | No. males fertile | Percent fertile | Average no. of progeny per male | Factors deficient |
|---|---|---|---|---|---|
| L41 | 2948 | 14 | 0.47 | 10.93 | *kl-3* |
| L19 | 2765 | 9 | 0.32 | 1.56 | *kl-3–5* |
| L34 | 8881 | 68 | 0.76 | 11.31 | *kl-5* |
| L3 | 5334 | 67 | 1.26 | 17.30 | *kl-5* |
| L28 | 2802 | 20 | 0.71 | 5.60 | *kl-5* |
| L36 | 7030 | 72 | 1.02 | 8.54 | *kl-4–5* |

tested further is that these chromosomes have localized damage with inactivation of the adjacent fertility genes by position effect. The consequences of this position-effect inactivation are the same as those for loss of the genes by deletion and do not alter the conclusions drawn from the complementation studies in any way. One consequence of this phenomenon was that some of these cultures that produced very few progeny were at first classified as fertile and yielded discordant results between reciprocal combinations of the same two sterile Y's. Retests showed that these were not cases of true complementation.

Other discordant results between reciprocal combinations of the same two sterile Y's turned out to have quite a different explanation. In these cases, an unmarked fertile Y was recovered from the progeny males. Since no unmarked fertile Y is used in any of the stocks or crosses associated with the fertility tests, and since the presence of other markers ruled out contamination, some other explanation for these Y's had to be sought. Further tests indicated that the unmarked Y's could have been the result of crossing over between the sterile Y and the $Y^L$ attached to the centromere of the XY chromosome (BROSSEAU 1958). The frequency of this type of exchange is sufficient to explain the cases found in the fertility tests.

*Results of crosses with attached—X detachments*

It is well known that both induced and spontaneous detachment of attached—X chromosomes frequently involves one arm of the Y. The common procedure in characterizing these detachments is to test them against one or the other arm of the Y to determine whether the detachment carries one of the fertility complexes. In the preceding section, however, it was shown that a deficiency of one of the genes in each of the complexes is generally as effective in sterilizing the male as loss of the whole complex; thus deficiency of any one of the genes will look like loss of its entire complex. With the sterile Y's, it is possible to demonstrate the presence of part of one of the complexes on some of the detachment products. The usefulness of such a demonstration is evident from the following considerations. (Details of this discussion can be followed in Figure 2.) The exchange pictured involves $Y^L$; however, exactly the same argument applies to exchanges with $Y^S$. The detachment can be visualized as a heterochromatic

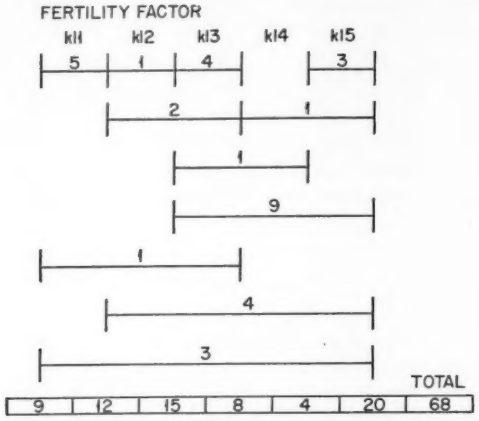


FIGURE 1.—Complementation map for the fertility region of the long arm of the Y chromosome. Figures above the lines, representing the genetic length of each deficiency, indicate the frequency with which that deficiency was observed. The numbers at the bottom of the figure give the number of breaks that occurred between each fertility gene.

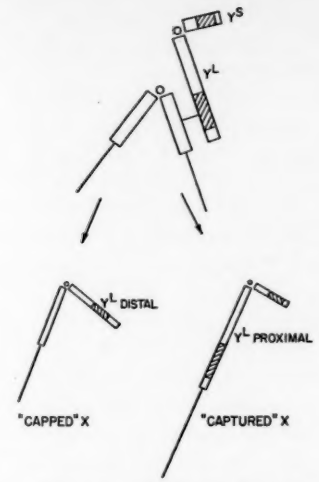


FIGURE 2.—The origin of the detachments of an attached-X chromosome where the detachment involved an exchange between the base of one of the X's and $Y^L$. The crosshatched areas represent the region of the fertility complexes.

exchange between the arm of the Y and the proximal heterochromatin of one of the X's. By the terminology of ABRAHAMSON, HERSKOWITZ and MULLER (1956), the detachment product that carries a Y centromere with the X arm attached is a "captured" X and the reciprocal product is a "capped" X. If the location of the exchange in the Y arm is within the limits of the fertility complex, the resulting captured X's will carry the proximal portions of the complex and the capped X's will sample the distal parts of it. The detachments can be combined with known deficient Y's and the males tested for fertility to determine which of the genes are proximal and which are distal.

The detachments used in these studies were kindly supplied by DR. D. R. PARKER (PARKER and HAMMOND 1958), who induced them by X-rays. Five of 13 captured X's with an exchange located in $Y^L$ carried a portion of the fertility complex. The results of the tests with these detachments are given in Table 6. These data show that *kl-1*$^+$ is the proximal-most gene. Further confirmation of this is found among the $Y^{KL-}$'s that had also lost the marker $y^+$, which is located at the end of the long arm. Sterile Y's 102 and 103 (see Table 4) simultaneously lose *kl-3–5* and $y^+$, and thus are located distally to *kl-1*$^+$ and *kl-2*$^+$. Only one of the 15 tested capped X's carried any portion of the fertility complex, but this portion was from the middle of the complex (*kl-2*$^+$ and *kl-3*$^+$) indicating that

TABLE 6

*Results of tests for $Y^L$ fertility factors on captured-X's from detachment of attached-X's*

| Detachment no. | Fertility factor deficiency† | | | | |
|---|---|---|---|---|---|
| | *kl-1* | *kl-2* | *kl-3* | *kl-4* | *kl-5* |
| 108–7 | + | + | + | + | — |
| 108–43 | + | + | — | — | — |
| 110–10 | + | + | + | + | — |
| 129–30 | + | — | — | — | — |
| 129–34 | + | + | — | — | — |

† +,Covers deficiency and —, does not cover deficiency.

the detachment involved a complex event. These results place *kl-1*$^+$ at the proximal end of the fertility region and to a certain extent confirm the order of the genes found in the complementation studies.

A similar analysis was carried out with detachments involving $Y^S$. Only two captured X's of this type were available; however, one of these carried *ks-1*$^+$ indicating that this factor is proximal to *ks-2*$^+$. In the capped X's where $Y^S$ is the cap and the exchange is located within or distal to the fertility region, it is not possible to state positively that the Y is involved in the detachment. The other chromosomes can be ruled out by means of genetic tests with the possible exception of the very tips of the autosomes; it is therefore likely that this sample of "unidentifiable" detachments consists mostly of $Y^S$ caps. Ten out of 37 of these detachments gave evidence of $Y^S$ involvement. Two of these ten carried *ks-2*$^+$ alone, confirming its distal position. The remaining eight detachments are fertile with single gene deficiencies for *ks-1*$^+$ and *ks-2*$^+$ but are sterile with $sc^{8L}Y^L$ and with a double deficiency for *ks-1*$^+$ and *ks-2*$^+$. These detachments were also fertile with $Y^{c1}$. These results suggest that a third factor is present, probably on the short arm, and is not represented as a single gene deficiency in the sample of sterile Y's. If a third factor exists then it must be located between *bb*$^+$ and the centromere since $Y^{c1}$ lacks *bb*$^+$ but may contain a short proximal portion of $Y^S$. BAKER's results (1955) indicated that *KS* is located distally to *bb*$^+$. The failure to recover this possible third factor by itself may be attributable to the selection exercised against the sterile Y's that did not disjoin normally from the X because the pairing sites are probably also located in this region. There is also a possibility that this is the factor shared by the X and the Y, the existence of which was suggested by NEUHAUS (1939). It cannot be stated definitively that this factor is on the $Y^S$ and not the $Y^L$ side of the centromere, although it is most likely very close to the centromere.

### *Spermiogenesis*

Aceto-orcein squash preparations of the sterile males were made for each of the sterile Y's to determine if the various genes could be distinguished from one another on a phenotypic basis. The testes from five males for each genotype, including several X/0 males, were examined. In no case was any phenotypic distinction

possible with the possible exception that the sterile Y's produced a slightly more normal picture than the X/0 males. The X/0 males do not seem to proceed quite so far in sperm maturation as the males with a sterile Y, but this difference is of doubtful significance. In all cases meiosis seems to be completely normal as do the early stages of spermiogenesis. The breakdown apparently occurs at the time of sperm elongation and maturation. Few fully elongated sperm were found, and then these were never motile. The vasa efferentia were usually empty (the presence of a very few sperm would not have been detected in this examination). Thus it has not been possible to distinguish between the fertility genes phenotypically.

## DISCUSSION

The fertility region of the Y chromosome was studied by NEUHAUS (1938, 1939), who induced a series of Y-4 translocations that produced only sterile males in the absence of another free Y. His method was to produce males that carried the proximal portion of one translocation and the distal portion of another. He tested these males for fertility. This test is based on the assumption that the translocation carries a mutant fertility gene at the site of the translocation. If the breakpoint of the fragment is located proximal to the breakpoint of the translocation from which the proximal piece is drawn, then the mutation will be covered by the normal genes and the male will be fertile. The opposite is true if the mutation is distal to the breakpoint. A low probability nondisjunctional event is necessary to produce the proper male for testing. Because this male is phenotypically indistinguishable from his nonfragment-bearing brothers it was necessary to test large numbers of males.

NEUHAUS concluded that there were at least four fertility genes on the long arm of the Y and five on the short. These results are at variance with mine. The difference for $Y^L$ is slight (four *vs.* five) and is probably attributable to the small number of $Y^L$-4 translocations available to NEUHAUS. The difference for $Y^S$ is not so easily explained (five *vs.* two) but is probably the consequence of the different methods used. NEUHAUS could not be sure that he had a male of the right genotype in his test sample, which could have resulted in misclassification. Other errors are also possible. Careful examination of Table 3 of the NEUHAUS paper (1939) reveals several inconsistencies in the results of the fertility tests, which tends to rob the work of any real meaning.

The nature and function of the fertility genes is far from clear at present. All the genes seem to be producing the same effect as evidenced by the similarity of their effects on spermiogenesis, yet each must differ in function as loss of one gene is phenotypically equivalent to loss of the whole set. Superficially, it seems as though each complex is one functional unit; however, the complete complementarity of the mutants indicates that actually multiple-functional sites are involved. Another possibility is that the genes perform successive or related steps in some synthetic pathway. But in the complete absence of any information about the function of the fertility genes in a chemical or physiological sense, it is

not possible to say anything further about this idea—an idea that might have some implications of one possible origin of these genes. The fertility genes could represent the end product of genetic duplication and subsequent divergence of function such as that postulated by SEREBROVSKY (1938) for *ac* and *sc*; there is no experimental evidence for or against this idea.

Several hypotheses have been suggested for the origin of the Y chromosome in Drosophila (MULLER 1918, 1932; HALDANE 1933; NEUHAUS 1939). These hypotheses all have in common the idea that the Y is some kind of degenerate homolog for the X that has resulted from enforced heterozygosis and lack of crossing over. It is beyond the scope of this paper to discuss the genetic inertness or activity of heterochromatin except to point out that the failure to associate a discrete function with a portion of the genome cannot be considered as definitive evidence that this material has no function. The conclusion that the heterochromatin is inert is the result of asking whether it is essential to the individual organism. Often the answer is no. However, the error may be that this is the wrong question. Perhaps we should ask whether heterochromatin is important to the species or population. To this question, we have few answers. The further the "functionless", inert materials of the Y are investigated, the more functions become apparent.

The Y has no genetic regions homologous to most of the X and the view may also be taken that the X has no homolog for most of the genes or known genetic activities of the Y. This view is diametrically opposed to the notion that the Y is a degenerate X. From this new view the Y is not protected from natural selection by the X, but is constantly haploid and therefore constantly exposed to the forces of selection.

If the Y is a degenerate X, then some residual homology between the X and the Y should be found and, as pointed out earlier, this is the case. A portion of $Y^S$ is clearly homologous to a portion of the basal part of the X. However, $Y^S$ has an additional region, the fertility complex, which is not represented on the X. If an additional fertility factor that is shared by the X and the Y exists, the genetic data place it in the $Y^S$ region of X homology not in the fertility complex. There is no good evidence that any of the $Y^L$ has any counterpart on the X. The location of $bb^+$ has some bearing on this question, for, if $Y^L$ contains a mutant allele of *bb* (NEUHAUS 1939) or if $bb^+$ is really on the long arm (the evidence against this latter possibility is rather strong but not conclusive, see COOPER 1959), then $Y^L$ does have some homology with the X. The fact that all of the $Y^L$ deficiencies are $bb^+$ and that the only sterile Y lacking $bb^+$ is also deficient for the $Y^S$ fertility genes, argues that $bb^+$ is on $Y^S$. The *bb*-Df sterile Y also does not cover a lethal *bb*-Df, which indicates that $Y^L$ (presumed to be completely normal in this chromosome) does not carry even a mutant *bb*. It seems likely that the only region of the Y that is homologous to any of the X is the proximal portion of $Y^S$.

It is obvious that the genetic properties of the Y would have the following characteristics. The genes should be sex limited in their usefulness, as the male fertility factors are. The Y genes might even be expected to be detrimental to

the female, and, indeed, some of them are as evidenced by the loss of fertility in females with extra Y's. The detrimental effects of Y-chromosome hyperploidy could account for the relative stability of the Y in karyotype evolution in the genus Drosophila. Very few of the many chromosome interchanges that have occurred during the evolution of this genus have involved the Y (PATTERSON and STONE 1952), but if the Y is truly inert, many interchanges would have been expected. Only four species of Drosophila have dispensed with the Y altogether and are X/0 in the male. In these cases the essential material has probably been attached to some other chromosome (WHARTON 1944; PATTERSON and STONE 1952), but it is not clear how the potentially detrimental effects have been circumvented. In all the other species in the genus, the Y chromosome has been maintained by natural selection although in the laboratory it is not possible to demonstrate any phenotypic effect of the Y except on fertility. The existence of X/0 species demonstrates that the male fertility factors can be put onto the autosomes; thus these factors are probably not sufficient to maintain the Y as a separate entity. One possible exception is *D. busckii* where the X/0 male is lethal (KRIVSHENKO 1952). The lethality of these males is caused by the absence of the euchromatic short arm of the Y. This arm is derived from a fusion of the microchromosome (corresponding to the fourth chromosome in *D. melanogaster*) and the base of the X and the Y (KRIVSHENKO 1955).

Perhaps the best way to look on the Y chromosome is not as a degenerate chromosomal relic but rather as a unique, specialized element subject to and maintained as an entity by natural selection. The challenge is to uncover the genetic activities of this chromosome. The genetic definition of the fertility factors and the new genetic tools that they provide are a step in this direction.

## SUMMARY

The number and genetic organization of the male fertility regions of the heterochromatic Y chromosome were investigated by complementation studies with sterile Y chromosomes. The results indicate that there are at least two fertility genes on $Y^S$ and at least five on $Y^L$. The data for $Y^L$ are compatible with a linear order of the genes. In this respect this heterochromatic region is organized in a manner similar to euchromatin. Tests with detachments of attached–X's were the detachment has involved an exchange with Y have established the proximal-distal orientation of the fertility genes and confirmed the order of the genes found in the complementation studies. These results also suggest the presence of an additional fertility factor common to the X and the Y. Studies of spermiogenesis uncovered no obvious phenotypic differences in the action of the various fertility genes. It is hypothesized that the Y chromosome is a unique, specialized structure rather than an inert chromosomal relic.

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# AN ANALYSIS OF INBREEDING IN THE EUROPEAN BISON[1]

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DURING a study of inbreeding in man (SLATIS, REIS, and HOENE 1958) it became evident that the most striking consequences of inbreeding were increases in childhood death and in serious illnesses. It seemed worthwhile to investigate the death rates in the young of other large mammals. This would be of interest because large mammals fall into two sharply divided categories, (1) domesticated forms with a long history of inbreeding and artificial selection, and (2) wild forms none of whose ancestors have been subject to artificial selection.

It is known that some lethal genes exist in domesticated animals and one might predict that those species that have not undergone much inbreeding and artificial selection would have a higher frequency of recessive lethal genes. To gather information about wild species, data were sought from zoological gardens. The only satisfactory data appear to be for the European Bison, a species with no history of artificial selection, although with small population sizes in recent centuries.

## MATERIALS AND METHODS

### *The pedigree books*

The European Bison (occasionally known by its German name, *Wisent*), *Bison bonasus* (L.), had dwindled to two small populations by the middle of the nineteenth century. One subspecies, *Bison bonasus caucasius* Grevé, was limited to a region in the northwestern Caucasus Mountains, where it died out about 1930. The other subspecies, *Bison bonasus bonasus* (L.), was maintained in the forest of Bialowieza, Poland, until poachers extinguished the herd in 1922. The Caucasian subspecies was always completely wild, rarely even being observed. The herd at Bialowieza was carefully protected but was never subject to artificial selection. Within historical times, neither subspecies has been numerous.

The International Society for the Protection of the European Bison was established after the slaughter of the herd at Bialowieza and the near extinction of its largest daughter herd. Records were collected on the birth, death, and ancestry of the few living animals. These data were published in 1932 in the *Berichte der Internationalen Gesellschaft zur Erhaltung des Wisents*, and subsequent data have been published under this title (1932–1937) and more recently under the title of *Pedigree Book of the European Bison* (1949–1958). This paper will analyse the information contained in these volumes through the issue of 1958, which includes all data through 1954. The records give internal evidence of being carefully

[1] This work was performed under the auspices of the U. S. Atomic Energy Commission.

kept. Each animal, including stillbirths, is identified by a name and a number. For the purposes of this paper, only numbers will be quoted. These recording methods were adopted around 1924, and 553 animals born after 1 January, 1925 (animals with numbers above 140), have been used for analysis. Two other animals were omitted because of a lack of satisfactory information.

All living purebred European Bison are the descendants of a basic group of 17 animals whose parents are not accurately known. One of these (#100) was a bull born in the Caucasus Mountains in 1907 and brought to Germany as a calf. Its descendants represent the only Caucasian influence on the modern species, all of the other genes being derived from the Bialowieza herd. One female (#2) was born in the Berlin Zoo about 1883 of unknown parentage (presumably tracing back to Bialowieza). Seven animals (#85, 86, 89, 95, 96, 122, and 123) were taken from the herd at Bialowieza between 1903 and 1913. A daughter colony of the Bialowieza herd at Pszczyna, Poland (Pless, Germany, prior to 1919), was the birthplace of the other eight animals (#1, 7, 16, 32, 33, 42, 45, and 46), all born between 1881 and 1918. At least some of these Pszczyna animals (if not all) were more closely related to each other than to animals picked randomly from the Bialowieza herd. It is probable that most of the Pszczyna animals were inbred.

Although the Bison go back to 17 animals, their genetic constitution represents recombinations of only 12 diploid sets of genes. Animals #1 and 2 had an offspring, #3, which mated with #7 to produce #15, and these animals are represented in the modern species only through the descendants of #15. Similarly, one pair of Pszczyna animals (#32 and 33) and one pair of Bialowieza animals (#85 and 86) are represented through single offspring (#35 and 87, respectively). One other pair of Bialowieza animals (#122 and 123) is represented only through an inbred descendant (#147). Thus, the 213 living European Bison can present a maximum of 24 alleles at any locus, except as mutations may have increased this. The two indexes of inbreeding that will be used do not distinguish between pedigrees that go back as far as they can be traced and those that stop at the last ancestor to which two or more separate lines can be traced. Therefore, it is of no consequence whether the calculations are made with respect to the genes carried by animals #15, 35, and 87, as was actually the case, or their ancestors, #1, 2, 7, 32, 33, 85, and 86. However, because #147 was inbred, it is necessary to trace descent from #122 and 123. Thus, the species has been treated as derived from a "foundation herd" of only 13 animals (see Table 1), all of which were alive during the latter part of 1918 and most of 1919.

These 13 genomes are unevenly represented in the living animals. Table 1 indicates the proportion of the genes of the 213 Bison living on 31 December, 1954, which traces back to each of the foundation animals. Six animals come within 25 percent of the mean, 0.077. However, #46 contributed only about a tenth of this mean, and the combined contribution of #122 and 123 is equally small (five eights of this represents the genes of #122 and three eights, those of #123). Two other animals are just below half of the mean value.

Animals #42 and 45, survivors of the predations of poachers on the herd at

TABLE 1

*The genetic contribution of the foundation herd animals to the living species*

| Animal | Proportion |
|---|---|
| Born at Bialowieza | |
| (# 85 + 86) 87* | .072 |
| # 89 | .096 |
| # 95 | .038 |
| # 96 | .063 |
| #122 + 123 (147)* | .009 |
| Born at Pszczyna | |
| (# 1 + 2† = 7) 15* | .082 |
| # 16 | .091 |
| (# 32 + 33) 35* | .027 |
| # 42 | .188 |
| # 45 | .264 |
| # 46 | .009 |
| Born in the Caucasus Mountains | |
| #100 | .061 |

* The preceding animals are represented only through this descendant (see text). The 13 animals not in parenthesis formed the foundation herd.
† Born in the Berlin Zoo.

Pszczyna, founded a remarkably inbred herd by themselves in 1922. Their genes now represent 0.452 of the genetic constitution of the species (this value was about 0.50 when 17 animals, representing the major herd descended purely from this pair, were wiped out by foot-and-mouth disease in December, 1953). Twenty-nine living and 75 dead animals have no foundation herd ancestors other than #42 and 45, while only 52 living animals do not trace back to this pair through any ancestral line. No individual traces back to all of the foundation herd animals and only a few represent all but two of the 13, though many represent all but three. The Caucasian animal, though contributing only six percent of the genes of the species, is an ancestor of 70 percent of the living individuals, having almost as many descendants as #42 and 45. All of the other foundation herd animals are less frequently ancestors of the living animals, down to #122 and 123, who have only 18 living descendants (8.5 percent of the species).

### *Measures of inbreeding*

The standard coefficient of inbreeding, F (WRIGHT 1921), has been calculated for each animal. Similarly, for each animal a value has been calculated which is being designated as *ll*. This is the *likelihood of genetic death* if each ancestor in the foundation herd possessed a single recessive lethal gene, *l*, and if each of these lethals was at a different locus. For these purposes, a "lethal" gene will be understood to mean a gene which kills late enough in embryology to be recorded as a stillbirth, or which kills liveborn animals prior to the onset of reproductive maturity (about the second birthday, in Bison). Penetrance and recessivity are assumed to be complete. If a set of records were to include information on fertility, the frequency of genes causing sterility could also be computed.

Although *ll* is not a direct measure of the quantity of inbreeding in the ancestry of an animal, it may "measure as directly as possible the effects to be expected" from the observed inbreeding (WRIGHT 1922). On this definition, it seems reasonable to refer to *ll* as an inbreeding coefficient.

### *The characteristics of* ll

F and *ll* are both inbreeding coefficients, but they are only slightly related. When one is zero, the other must also be zero. If a given ancestor is assumed to have been *Ll* and leads through a separate and uncomplicated line of descent to each of the parents of a particular animal, the value of *ll* will be half of the F value due to this same ancestor, since F measures homozygosity for *L* and for *l*, but *ll* measures homozygosity for the latter only. However, if the lines of descent are not separate or if one or more of the intermediate ancestors is also inbred with respect to the given ancestor, then the value of *ll* will be less than half that of F. Several examples of the calculation of *ll* will aid in understanding this.

Figure 1 shows the known ancestry of #220. Animal #173 has an F value of

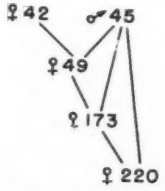


FIGURE 1.—The pedigree of animal #220.

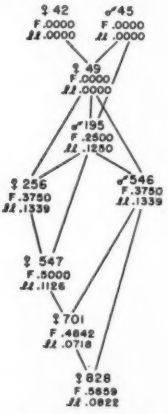


FIGURE 2.—The pedigree of animal #828.

0.25 and a *ll* value of 0.125. Animal #220 has an F value of 0.375 and a *ll* value of 0.1429. The calculation of the *ll* value was performed in the following manner. We assume that animal #42 is heterozygous for a lethal at one locus and that #45 is heterozygous for some other lethal. Since #220 can be homozygous only for the lethal in #45, the lethal carried by #42 will not be considered. We know that the probability that #173 would have been homozygous for any lethal carried by #45 is 1/8. Since #173 was a viable animal, it was not homozygous for the lethal, and so had 4/7 probability of being heterozygous and 3/7 probability of being homozygous normal. Thus, #220 had 1/4 of 4/7 probability of being a homozygote, and her *ll* value is 1/7, or 0.1429.

The fact that there is not a direct relationship between F and *ll* values may be seen from Figure 2, which indicates these values for the pedigree of #828, a highly inbred descendant of #42 and 45. It will be instructive to observe the calculation of the *ll* values of #256 (and also #546, her full brother), for which F = 0.375, *ll* = 0.1339. Her sire, #195, had 1/8 probability of being homozygous for a lethal carried by #45. As a viable animal, he would carry such a lethal 4/7 of the time. Only in half of these instances would her dam, #49, have been carrying the lethal also, and #256 would be *ll* 4/7 times 1/2 times 1/4, or 0.0714. We must add to this the probability of the lethal carried by #42 being homozygous in #256, which is 1/8 of the 1/2 chance that #49 is *Ll*, which is 1/16, or 0.0625. Therefore, the total *ll* value for #256 is 0.1339. Similarly, the *ll* values for #547, 701, and 828 are compounds of the *ll* values for lethals carried by both #42 and 45. It is clear in this pedigree that an increase in F can be accompanied by a decrease in *ll* and that the changes are not closely correlated.

For small values of F, there is a definite correlation with *ll* in these data. However, for the 199 animals with F equal to or greater than 0.2500 (this point being chosen arbitrarily as the lowest F value for which any observed *ll* value exceeded 0.10), there is actually a significant negative correlation between F and *ll*, r being −0.205.

It will be observed that as a measure of inbreeding, *ll* is defined as the *sum* of the *ll* values for each ancestor taken separately. Thus, in the example given above, the *ll* values were calculated separately for descent from #42 and from #45 and these two values were added together. In practice this is by far the simplest thing to do but it is not quite accurate. The true value of *ll* in the above example is 0.1295, rather than 0.1339, as can be computed by designating #42 as *AaBB* and #45 as *AABb* and assuming either homozygous recessive to be lethal. In the example of Figure 1, if #45 had himself been the offspring of two animals of the foundation herd, then the *ll* value of #220 as calculated in this paper would still be 0.1429, but the true *ll* value would be 0.1450. The difference between the summed *ll* value and the true *ll* value is dependent upon the occurrence within the pedigree of ancestors who might have been inbred for lethals from a foundation herd animal. As can be seen from these two examples, the true value may be either greater or less than the summed value. The calculation of the true *ll* value often is excessively complex. For this reason, the technique of summing the separate *ll* values has been adopted. The discrepancy that results is never very large. The following paragraph pertains only to the summed *ll* values, rather than to the true values.

The maximum value of *ll* will occur after repeated backcrossing to a heterozygous ancestor. In this situation, *ll* approaches 0.1464 asymptotically (F approaches 0.5). The beginning of such a pedigree is shown in Figure 1. As the intensity of inbreeding increases in inbreeding other than backcrossing, F approaching 1.0, lethal genes become infrequent. The *ll* value will be only a small fraction of the F value, as can be observed in Figure 2. The maximum value of *ll* in a nonbackcrossed organism will occur in the mating of sibs whose parents

are of unrelated, outbred stocks. In this instance, $ll = 0.125$ ($F = 0.25$). Larger $ll$ values occur in organisms in which self-fertilization is possible, but special cases will not be discussed here.

As has been noted, the $ll$ value assumes that the genes are completely penetrant with respect to lethality. If one were to calculate the mortality produced in a pedigree such as that shown in Figure 1 by a gene which killed half of the individuals homozygous for it, the $ll$ value for #173 would be 0.0625, or half of the value under complete penetrance, but the value for #220 would be 0.0833, or more than half of the value under complete penetrance. This example demonstrates the general rule that for pedigrees in which there are no inbred ancestors, incompletely penetrant genes produce mortality equal to the $ll$ value multiplied by the penetrance. (This is true for F as well as for $ll$ and this might be the reason for the usefulness of the theory of lethal equivalents in the uncomplicated human pedigrees studied by MORTON, CROW, and MULLER (1956). In that paper, F was the inbreeding coefficient ostensibly employed, but it was modified to be identical with $ll$.) The example given above also demonstrates that if at least one inbred ancestor occurs in a pedigree, the mortality produced by a semilethal gene will be greater than the $ll$ value multiplied by the penetrance of the gene. The precise effect of this will depend on the complexity of the pedigrees and the distribution of semilethal genes in the population.

Thus, semilethal genes will have a serious effect on the $ll$ calculations. In relation to $ll$ values they will contribute to lethality in a variable manner, rather than in a manner directly related to their penetrance. This will tend to raise the estimate of the number of lethal equivalents carried by the average foundation herd ancestor. There is no simple way of correcting for this effect. However, the difference between F and $ll$ will be shown in the discussion to have a bearing on this problem.

### *Treatment of the data*

Each animal has been classified according to its life span. There are three categories, (1) perinatal death, which includes stillbirths and deaths within one month of birth, (2) juvenile death, which includes all deaths before the second birthday other than those in category (1), and (3) survival past the second birthday.

The records list 119 animals that were not inbred (F and $ll$ are zero). Of these, 15 died in the perinatal period. However, two animals probably died because of foot-and-mouth disease. These two deaths were essentially nonselective in nature, since it appears that this disease usually kills all of the animals in a stricken herd. Other foot-and-mouth deaths plus many deaths associated with the end of World War II have also been classified as nonselective. Statistically, it seems appropriate to treat the young animals that clearly suffered nonselective deaths as if they had not died at all. Within any period prior to maturity, their life experience would approximate that of half as many animals surviving the period (i.e., two calves dying of clearly nonselective causes within the perinatal period would roughly

represent the same experience of life as one calf that survived this period). Thus, the outbreds are treated as if there had been 13 deaths (all deaths not classified as nonselective) among 119 minus half of two, or 118 animals, for a rate of 0.1102 perinatal deaths. There were 104 animals that survived to one month of age and of these, eight might have died of selective factors and four clearly died of the afore-mentioned nonselective causes. In addition, one outbred animal is less than two years of age. The four nonselective deaths and the animal which has not yet completed the age period are treated identically, half being classified as having survived the period, so that there are eight deaths among 104 less 5/2 animals (8/101.5), or 0.0788 juvenile deaths. Each class of inbred animals has been treated in the same manner. The frequencies of animals surviving the perinatal and juvenile periods have been shown in Table 2 according to their degree of inbreeding.

## RESULTS

For reasons to be noted in detail in the discussion, it is important to discover the relationship between degree of inbreeding and premature death. For this purpose, it was decided to calculate the change in death rate as the degree of inbreeding changes, which is the regression of death rate on degree of inbreeding. This regression has been calculated for (1) the total population as classified by F, (2) the total population as classified by *ll*, (3) the total population as classified by *ll* except for the omission for each animal of any contribution to *ll* due to descent from #100 (the Caucasian bull), and (4) only that contribution to *ll* due to descent from #100. Because F measures homozygosity for normal alleles as well as for lethals, it is necessary to double the value of the regression of death rate on inbreeding as calculated for F to make these results comparable with the calculations based on *ll*. The values cited in Table 3 have been corrected in this manner.

These regression coefficients have been calculated separately for perinatal and for juvenile death. In each of these categories the calculations have been performed in two manners. The first of these is a least squares estimate made with the data of Table 2. A standard error of this estimate may be obtained as $\frac{\sigma y\sqrt{1-r^2_{xy}}}{\sigma_x\sqrt{N-2}}$. The value of $\sigma y$ can be assumed to be $y(1-y)$, which is the standard deviation of a proportion. Since the correlation between $x$ and $y$, $r_{xy}$, is assumed to be small (but is not assumed to be zero), the second term in the numerator can be disregarded as not being very different from one. The terms in the denominator are easily obtained from the data. The standard errors indicate that a significant relationship between death rate and degree of inbreeding exists for all categories except (1) perinatal death as measured by F and (2) juvenile death as measured by *ll* when excluding the contribution due to descent from #100.

A second estimate of the regression is based on a pooling of all the data for inbred individuals at their mean value. The regression is calculated from the change in the frequency of death over the difference between outbreeding (F and *ll* as defined for the category equal zero) and the mean level of inbreeding.

TABLE 2

*Relationship between degree of inbreeding and frequency of early death*

| Mean F value | 0.0 | .025 | .075 | .125 | .175 | .225 | .275 | .325 | .375 | .425 | .475 | .525 | .575 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Perinatal death | | | | | | | | | | | | | | | |
| Frequency | .1102 | .0000 | .1467 | .1649 | .1000 | .0426 | .1458 | .2162 | .0962 | .1667 | .0952 | .1111 | .0000 | | |
| n | 118 | 3 | 75 | 48.5 | 60 | 47 | 48 | 37 | 52 | 30 | 21 | 9 | 3 | | |
| Juvenile death | | | | | | | | | | | | | | | |
| Frequency | .0788 | .5000 | .1789 | .1316 | .1373 | .0563 | .2000 | .1091 | .0909 | .2174 | .0645 | .1667 | .4000 | | |
| n | 101.5 | 2 | 61.5 | 38 | 51 | 35.5 | 35 | 27.5 | 44 | 23 | 15.5 | 6 | 2.5 | | |
| Mean *ll* value | 0.0 | .015 | .025 | .035 | .045 | .055 | .065 | .075 | .085 | .095 | .105 | .115 | .125 | .135 | .145 |
| Perinatal death | | | | | | | | | | | | | | | |
| Frequency | .1102 | .1053 | .1071 | .1212 | .1739 | .0222 | .0909 | .1190 | .1852 | .0500 | .2308 | .1667 | .1818 | .0000 | .0000 |
| n | 118 | 19 | 28 | 33 | 80.5 | 45 | 55 | 42 | 27 | 20 | 39 | 24 | 11 | 9 | 1 |
| Juvenile death | | | | | | | | | | | | | | | |
| Frequency | .0788 | .2222 | .1818 | .0741 | .1440 | .1316 | .1176 | .2154 | .2000 | .0571 | .1111 | .2500 | .0000 | .0000 | .0000 |
| n | 101.5 | 13.5 | 22 | 27 | 62.5 | 38 | 42.5 | 32.5 | 20 | 17.5 | 27 | 20 | 9 | 9 | 1 |

TABLE 3

*The regression coefficient of death rate on degree of inbreeding*

| Index of inbreeding | Population | Perinatal death | | Juvenile death | |
|---|---|---|---|---|---|
| | | Least squares ± S.E. | Outbred *vs.* inbred | Least squares ± S.E. | Outbred *vs.* inbred |
| F* | Total | .024 ± .060 | .137 | .140 ± .068 | .505 |
| *ll* | Total | .320 ± .120 | .252 | .283 ± .135 | .930 |
| *ll* | Exclusive of descent from #100 | .266 ± .120 | .344 | .155 ± .135 | 1.052 |
| *ll* | Descent only from #100 | 2.526 ± .722 | —.628 | 4.992 ± .834 | 13.723 |

* All values in this row have been doubled.

In addition to these two methods, an estimate of each regression has been obtained by use of the method of maximum likelihood, as employed by MORTON, CROW, and MULLER (1956). This method gives a somewhat more exact answer than is given by the usual method of least squares. However, the data are transformed in a manner which tends to make the regression coefficients as determined by the method of maximum likelihood greater than by the method of least squares. Because of the ease of calculating a standard error with the least squares method, the maximum likelihood results have not been used and are not given.

With the exception of one pair of twins, the Bison have produced only single-born offspring in captivity. The measure of female fertility was to assume that one birth is possible for each completed year of life after the third. Only a few animals that have been observed have lived long enough to pass out of the fertile period, and on the whole, the females have been kept in situations where yearly breeding was always possible. The outbred females averaged 0.4638 births per year in this fertile period and the inbred females averaged 0.4623 births. Obviously there is no evidence for a loss of fertility among inbred females. Similarly, there appears to be no evidence for a dependence of early mortality on the inbreeding of the dam. In fact, perinatal deaths had a frequency of 0.150 among the offspring of outbred females but only 0.098 among those of inbred females. Juvenile deaths dropped from 0.130 among the offspring of outbred females to 0.123 among those of inbred females.

## DISCUSSION

Most geneticists believe that of the deleterious recessive mutations arising in a population, some are approximately neutral in heterozygous condition. Other genes that have a deleterious recessive effect exist in populations because they are favorable in heterozygotes. However, there are at present two conflicting schools of thought concerning the usual effects of heterozygosity and homozygosity. One group believes that the vigor of crossbred individuals is due largely to the covering of harmful recessive genes (dominance theory). The other group believes that at many loci the possession of two different alleles is advantageous over being homozygous for the best available allele (overdominance theory). CROW (1952) has discussed the evidence for each of these points of view.

Where inbreeding is complex, there is not a strong correlation between F and *ll*. These measures of inbreeding may aid in distinguishing the two possible causes of the effects of heterozygosity. F is a measure of the increase in homozygosity after the initiation of inbreeding. Although the use of F is not related to overdominance theory, if it is correct that loss of heterozygosity will be related to loss of vigor and therefore, to early death (overdominance theory), then F should be a relatively direct index of early death. On the other hand, *ll* is a measure only of the homozygosity of genes that are being heavily selected against. Therefore, *ll* will be a relatively direct index of the early death that is due to the action of recessive lethal genes (dominance theory).

In addition to the above, difficulties introduced by the existence of semilethal

genes are differentiated by F and *ll*. Strong semilethals would behave much like completely penetrant lethals, and so would agree in their effects more with the *ll* value than with the F value. Semilethal genes of low penetrance would, essentially, be responsible for a lowering of viability of animals with a high F (and a relatively low *ll*) value. The existence of genes of this type in moderate numbers would be a major mechanism contributing to the apparent success of F as a predictive index of viability.

In Table 3, one may observe that there is a positive relationship between inbreeding and early death in the European Bison. It is reasonable to assume that this relationship is real, being another instance of the deleterious effects of inbreeding. If one of the indexes of inbreeding is more closely correlated with early death than is the other, it will suggest that the chief mechanism behind the inbreeding-death relationship is through the theory that is represented by the appropriate index.

The regression coefficients in Table 3 have been estimated in two ways. One method has been to compare the average increase in frequency of death with the change from outbreeding to the average amount of inbreeding, and the other method has been to calculate the regression of frequency of death on degree of inbreeding. A good index of inbreeding should order the animals in such a manner that these two methods would give very similar values. The only close approximation between these two methods is for perinatal deaths when ordered for *ll* value. The F estimates for perinatal death are relatively distant from each other. There is only a slightly better agreement between the *ll* estimates for juvenile death than between the F estimates.

These observations indicate that *ll* has, in one instance, described the relationship between inbreeding and death in a better manner than has F. Both *ll* and F have failed to describe the inbreeding-death relationship for juvenile deaths, largely because of the low death rate among the most highly inbred animals. As will be noted in the next few paragraphs, the most highly inbred animals are largely of a single strain which might by chance have had a very small number of recessive lethal genes. If, as seems possible from these pedigrees, highly inbred strains can be developed without appreciable loss of vitality, then it will become clear that dominance is the major mechanism of hybrid vigor. A similarly simple explanation of the low mortality of an inbred strain is not reasonable on the theory of overdominance.

The least squares fit of the *ll* data indicates that the average number of recessive genes causing perinatal death is of the order of 0.3 per foundation herd animal and a similar average number of such genes causes juvenile death. However, the uneven contributions of the various foundation herd animals to these calculations makes it advisable to break down the data according to the individual animals, where possible. The most interesting animal in this respect is #100, the Caucasian bull. He, representing a separate subspecies, might possibly have differed from the other animals to a great extent. Two separate tests have been performed in comparing this animal with the others. Firstly, the regression of frequency of perinatal and juvenile deaths on *ll* value has been recalculated

disregarding the contribution of #100. There has been a decrease in the slope of both lines, and particularly in the slope of the line for juvenile death. These results have been checked by a calculation of the relationship between death and that part of the *ll* value contributed by #100. The perinatal deaths show a slope of 2.5 and the juvenile deaths show a slope of 5.0. These values can not be considered as more than suggestive. However, they indicate that the Caucasian Bison almost certainly was heterozygous for genes that were lethal to homozygotes in both the perinatal and juvenile stages, and that the latter were more numerous.

Some of the animals born prior to 1925, and therefore not included in this analysis, independently confirm the presence of lethal factors in #100. The only offspring of #100 by one of his daughters died in the juvenile period, and of the four instances in which an offspring of #100 was mated with one of its own progeny, one died in the perinatal or juvenile period.

The small regression coefficients for the total *ll* values after elimination of the contribution of #100 make it difficult to observe any evidence for lethal genes among the other animals. Animals #42 and 45 have been studied for the apparent number of recessive genes that they carried. About half of the total *ll* value of the species is due to this pair. The regression of frequency of death on *ll* value are slightly positive: $0.105 \pm 0.119$ for perinatal death and $0.165 \pm 0.131$ for juvenile death. These small regression coefficients are not significantly different from zero. (In making these calculations for #42 and 45, only truly outbred animals were listed for $ll = 0.00$. In the last two rows of Table 3, all animals that are not inbred by the definition of the category are listed as having $ll = 0.00$. The method of analysis is different because inbreeding for #100 is positively correlated with early death and negatively correlated with inbreeding for #42 and 45.)

The regression of death rate on inbreeding will not be cited for any of the other animals. The *ll* values for descent from other foundation herd Bison are strongly correlated with *ll* values for descent from #100. However, the regression of death rate on *ll* values for the combination of all animals exclusive of #100 is not much different from that of #42 and 45 alone. This would indicate that the remaining animals collectively have only a minor relationship between death rate and inbreeding coefficient.

Reference to Table 1 will demonstrate that about 70 percent of the non-Caucasian genes of the living Bison are derived from the herd at Pszczyna. This herd was founded in 1865 by the importation of a bull and three cows from Bialowieza. One can assume that during the next few years the inbreeding was intensive. In 1893, another three bulls and two cows were imported from Bialowieza. The only other addition to the Pszczyna herd was the acquisition in 1900 of a bull that is thought to have been both the son and grandson of #1, who was himself a product of this herd.

The inbreeding in the Pszczyna herd may have done much to eliminate the lethal genes carried by the Bison of Bialowieza. The Pszczyna herd numbered about 70 animals during the 1890's. The animals added at that time would not

have changed the composition of so large a herd drastically, though they would have increased the average number of lethal genes per animal above the level to which it had fallen through inbreeding. It would seem that #42 and 45 (born about 1904 and 1917, respectively) may have been free of lethal genes. In addition, Pszczyna-born animals #1 and 16 are each recorded as having had three viable offspring by one of their own offspring, indicating that neither of them was carrying a large number of recessive lethal genes.

Unfortunately, satisfactory records do not exist for an evaluation of the average number of recessive genes carried by the animals listed in Table 1 as born in the Bialowieza herd. Most of their inbred descendants are also heavily inbred for genes from animal #100. Records exist of 11 highly inbred descendants of the Bialowieza animals (F at least 0.25, *ll* at least 0.10), all born by 1926, and of these, six died in the perinatal period. Thus, the number of lethal genes carried by individuals taken from the herd at Bialowieza was not great enough to preclude the survival of some highly inbred descendants. These results are consistent with an estimate that the average Bialowieza animal was carrying of the order of half a dozen fully penetrant recessive lethal genes or lethal gene equivalents.

It is clear that the process of mutation must be such that new mutants are being formed in each species with great regularity. The completely recessive mutants that are unfavorable in homozygous condition will be selected against in a manner which keeps their frequency roughly constant. In a small population, such as the ancestral populations of these Bison, there undoubtedly were many potentially mutant loci which were not represented by mutant alleles at any given time. However, for all such loci taken collectively, the Bison undoubtedly carried sufficient mutants to give the average animal a number of recessives. The data for the Caucasian animal estimate (with little accuracy) that he carried of the order of seven recessive lethal genes. The Bialowieza animals probably had fewer than this number. The Pszczyna animals obviously had very few recessive lethal genes. A remarkably high intensity of inbreeding in the first years of the Pszczyna herd might explain its later lack of lethals. Furthermore, because the number of recessive genes per animal varies randomly, there is the possibility that the original bull sent to Pszczyna fortuitously had a small number of lethal genes. This would help explain the success that this herd had in starting with small numbers while other small herds did not thrive and survive.

It is of interest that the average number of recessive lethal genes carried by a wild Bison in the forests of Poland or the Caucasus Mountains appears to have been about six and this number is similar to the number of recessive genes found to cause death or serious illness in man (SLATIS, REIS, and HOENE 1958).

## SUMMARY

The European Bison provide data which can be utilized for a study of the effects of inbreeding. At the end of 1954 the species contained 213 living animals but it can contain at most only 24 alleles at any locus, exclusive of very recent

mutations. A small proportion of the genetic ancestry of the species traces to a subspecies which lived in the Caucasus Mountains, a larger proportion traces directly to a large herd in Poland, and most of the genes appear to have passed through an inbred subline of the Polish herd.

A new index of inbreeding, *ll*, has been devised. The effects of inbreeding as measured by this index were compared with the effects as measured by the standard index of inbreeding, F. The relationship between the degree of inbreeding and the frequency of perinatal and juvenile death was found to be slightly greater for *ll* than for F. The two indexes of inbreeding treat the data differently in that *ll* measures the effect of homozygosity for recessive lethal genes while F measures the loss of heterozygosity. It appears from this analysis that the deleterious effects of inbreeding may be due more to the presence of recessive lethal genes in wild, outbred ancestors than to the need of the individual to maintain a high level of heterozygosity; that is to say, that dominance rather than overdominance may be the main mechanism of hybrid vigor in these mammals.

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# CHRONIC GAMMA IRRADIATION EFFECTS ON THE MUTABLE V AND STABLE R LOCI IN A CLONE OF NICOTIANA[1]

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THE origin and genetic analysis of a Nicotiana culture showing flower color variegation was described by SMITH and SAND (1957). This somatic instability was interpreted to result from the intrinsic reversible mutation of two alleles of the gene *V*. Phenotypic variability in this material is enhanced by sensitivity of the variegation to environmental changes (SAND 1957). Since the culture also contains stable genes for flower color, it provides material for the study of interaction between (1) environment and (2) unstable and stable genes.

Data are presented below for both somatic and germinal effects of chronic gamma irradiation on a mutable allele of the *V* gene and on the stable *R* gene in a clone of Nicotiana. It is shown for low dosage rates that somatic response of the V-system is tenfold greater than that of the R-system, but that the germinal response detected in the progeny is grossly similar for the two systems. The reasons for these differences are of considerable theoretical interest, and may yield some important clue to differences between mutable and stable genes. Parts of this work have been summarized previously (SAND, SMITH and SPARROW 1954; SAND 1959).

## MATERIALS AND METHODS

Clone S5230–5, employed in this study, was propagated from a single sectorial plant of the Variegated-3 culture. This culture has been selected following the same interspecific cross in Nicotiana which gave rise to the Variegated-1 culture. Genetic analysis for sectorial individuals ($v_s/v_s$) of Variegated-1 has been pre-

[1] This work is a joint contribution from three institutions. It is published as paper number 319, Department of Plant Breeding, Cornell University, where the work was begun while the senior author was a graduate student with HAROLD H. SMITH, then Professor of Plant Breeding at Cornell. Irradiation treatments were done during this same period with the sponsorship of ARNOLD H. SPARROW at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission. The somatic response analysis was submitted by the senior author in 1955 as part of a thesis presented to the Faculty of the Graduate School of Cornell University for the Degree of Doctor of Philosophy. Progeny tests and the analysis were completed and the final manuscript was prepared at The Connecticut Agricultural Experiment Station.

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sented by SMITH and SAND (1957). Genetic interpretation of the present study is based on analogy of Variegated-3 with Variegated-1. However, a distinction is maintained by substituting $v_{S3}$ for $v_S$ in designating the unstable dominant allele associated with variegation. The clone is heterozygous for two genes affecting flower color: $v_{S3}/v_s$ and $R/r$. Segregation data for the latter gene have been published by SMITH (1937).

The stable *R* gene conditions red anthocyanin pigmentation in the epidermal cells of the petals. Individuals of $r/r$ genotype have purple anthocyanin. Since *R* is dominant, members of the heterozygous clone have normally red flowers. Purple somatic sectors should occur in $R/r$ individuals if (1) the gene *R* is deleted, mutated to *r*, or otherwise inactiviated, or if (2) the physiological system related to *R* is altered or inactivated. Such purple sectors are rare in flowers of S5230–5 under usual conditions.

The allele $v_{S3}$ conditions uniformly solid-colored petal epidermis; $v_s$ results in speckled petals in which clusters of pigmented cells are scattered among a background of colorless cells. Because $v_{S3}$ is dominant, the heterozygote, $v_{S3}/v_s$, has solid-colored tissue; but due to events that resemble mutation of $v_{S3}$ to $v_s$ during petal development, the solid tissue contains sectors of speckled tissue. Such speckled sectors are frequent in flowers of S5230–5 even under normal cultural conditions. Flowers of this clone are illustrated in a previous publication (SAND 1957).

By counting purple and speckled sectors on irradiated plants, the somatic effect on a stable gene system can be compared with the effect on a mutable gene system in the same tissue. Similarly, germinal effects on the same genes may be studied by counting the number of purple and speckled individuals in progeny from irradiated plants.

Young rooted cuttings of clone S5230–5 were transplanted to the gamma field at Brookhaven National Laboratory on June 2, 1953. Cobalt$^{60}$ was used as the source of the irradiation, operating 20 out of each 24-hour period. The various levels of treatment were secured by placing the growing plants in the field at measured distances from the radiocobalt source, where they remained throughout the summer. More detailed information concerning the gamma field and its operation at Brookhaven is described in a publication by SPARROW and SINGLETON (1953).

From four to seven randomly chosen cuttings were subjected to each of the following 11 irradiation treatments: control (0.00003), 0.74, 1.13, 3.12, 6.05, 12.0, 24.9, 47.2, 105, 190, and 310 roentgens per 20-hour day.

Beginning July 7 on the 35th day of exposure, data were taken from the first ten levels of treatment for seven days. Two randomly selected flowers were harvested from each of two plants at each treatment every day. Using the technique described elsewhere (SAND 1957), the following data were taken on each of three petal lobes per flower: (1) the number of proximal points of speckled sectors in one square centimeter, and (2) the number of purple-sector proximal points in the same square centimeter.

Again, beginning August 19 after 78 days of exposure, data were taken from the first four treatment levels (omitting the 0.74 level) for seven days. During this scoring period nine flowers were harvested from each treatment each day. These flowers were scored for speckled and purple sectors in the same manner as is indicated above.

Data also were taken after 80 days of exposure to permit estimation of the average number of epidermal cells per square centimeter of petal area. Field notes were taken on the plants with respect to plant height, morphological abnormality, and seed-set. Open field pollinated seed was harvested where possible from all plants at each treatment level.

## RESULTS

### *General notes*

Plant height was approximately normal ($69 \pm 3.7$ cm) at dosages of 105 roentgens per day or less, but dropped to $59 \pm 5.8$ cm at 190r per day and to $52 \pm 3.8$ cm at 310r per day. Flowering was abundant at all treatment levels employed except 0.74, but the size of flowers was reduced at 310r per day. Petals and leaves showed some deformation at 105r per day, and these organs were drastically malformed at 310r per day. It was for this reason that flowers from the latter treatment level were not scored for sector frequency. In addition, plants at the 0.74 level were poorly located outside the gamma field fence and did not produce sufficient flowers for regular somatic scoring with the other treatments. No seed was set on plants at 310r per day, rather sparse seed was present at 190r per day, whereas abundant seed was set at 105r per day and lower levels of irradiation.

### *Somatic effects*

#### Statistical treatment

The somatic sectoring data are summarized in Tables 1 through 5 and Figure 1.

Tables 1 and 2 present the treatment means for data taken in July and August, respectively. Comparisons between the two periods of scoring may be made on the basis of treatments common to both: control through 6.05 roentgens per day.

For purposes of comparing the sectoring frequencies studied in somatic tissue with published rates of mutation in germinal tissue the former have been expressed as rates per 10,000 epidermal cells. An estimate of the number of epidermal cells in three square centimeters of petal area was obtained from data on cell dimensions. However, since only sectors eight cells or larger in size were scored, the effective cell population per three square centimeters was considered to be one eighth of this value or about 48,000 cells. This estimate was used in calculating the tabulated rates per 10,000 cells.

A combined analysis of variance was applied to the sector totals of Table 2 and the totals for the corresponding treatments of Table 1. Use of the totals in computation and use of the nonsignificant interactions as error should obviate

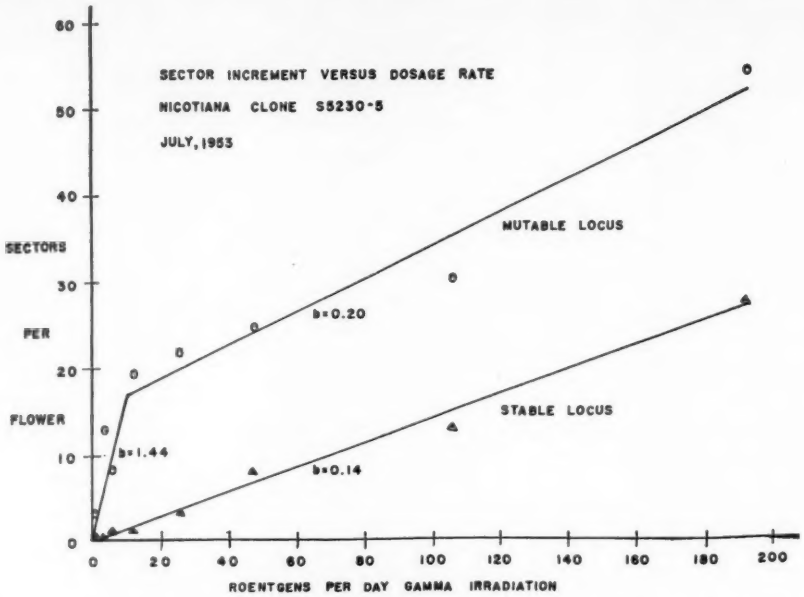


FIGURE 1.—Dosage-response curves showing the frequencies of somatic sectors induced by chronic gamma irradiation. Speckled sectors (associated with the mutable locus *V*) respond more sensitively in the low dosage range than purple sectors (associated with the stable locus *R*).

TABLE 1

*July, 1953 somatic sector frequency versus chronic gamma irradiation dosage*

| Dosage rate roentgens per day | Number of flowers scored | Total number of sectors counted* | | Somatic mutation rate† per $10^4$ cells | |
|---|---|---|---|---|---|
| | | Speckled | Purple | Speckled | Purple |
| 190 | 28 | 3,220 | 790 | 23.9 | 5.87 |
| 105 | 28 | 2,545 | 380 | 18.9 | 2.82 |
| 47.2 | 28 | 2,383 | 237 | 17.7 | 1.76 |
| 24.9 | 28 | 2,307 | 108 | 17.1 | 0.80 |
| 12.0 | 28 | 2,236 | 55 | 16.6 | 0.41 |
| 6.05 | 28 | 1,929 | 50 | 14.3 | 0.37 |
| 3.12 | 28 | 2,055 | 20 | 15.3 | 0.15 |
| 1.13 | 28 | 1,782 | 27 | 13.2 | 0.20 |
| 0 | 28 | 1,687 | 14 | 12.5 | 0.10 |
| Total 0–190 | 252 | 20,144 | 1,681 | 16.6 | 1.39 |
| Total 0–6.05 | 112 | 7,453 | 111 | 13.8 | 0.21 |

* Sector origin points counted in 3 $cm^2$ of petal area for each flower.
† Effective epidermal cell population scored in 3 $cm^2$ = 48,134 ± 3.5 percent cells.

TABLE 2

*August, 1953 somatic sector frequency versus chronic gamma irradiation dosage*

| Dosage rate roentgens per day | Number of flowers scored | Total number of sectors counted* | | Somatic mutation rate† per $10^4$ cells | |
|---|---|---|---|---|---|
| | | Speckled | Purple | Speckled | Purple |
| 6.05 | 63 | 3,252 | 78 | 10.7 | 0.26 |
| 3.12 | 63 | 3,895 | 33 | 12.9 | 0.11 |
| 1.13 | 63 | 2,796 | 25 | 9.2 | 0.08 |
| 0 | 63 | 2,642 | 14 | 8.7 | 0.05 |
| Total 0–6.05 | 252 | 12,585 | 150 | 10.4 | 0.12 |

* Sector origin points counted in 3 $cm^2$ of petal area for each flower.
† Effective epidermal cell population scored in 3 $cm^2$ = 48,134 ± 3.5 percent cells.

statistical difficulties due to the Poisson distribution of the very small individual counts of purple sectors. This analysis is summarized in Table 3.

Figure 1 presents the July dosage response curves for the two types of sectors plotted as *radiation-induced increments* above the control frequencies taken as a zero reference point. The purple-sector regression has been calculated as a least squares estimate of the best straight line for all treatment means. Although the fit is visually good, a valid test of its statistical significance is not immediately obvious because of the Poisson distribution. It is not helpful to employ a transformation of scale to normalize these data because the very process of taking the square roots of the ordinate values will destroy any original linear trend. Thus working in the original scale of measure, the treatment by days interaction was tested for Poisson distribution by partitioning into eight sets of mean differences. Since the eight averages of these sets of mean differences show no marked correlation with their respective within-sets variances, and since the variances are relatively homogeneous, the treatment by days interaction shows a normal rather than a Poisson distribution and was employed as error. Assuming that the deviations of the treatment sums from their mean and from their regression line

TABLE 3

*Analysis of variance of speckled and purple sector sums for July and August scoring from the lowest four irradiation treatments*

| Source | d.f. | Sum of squares | Mean square |
|---|---|---|---|
| Speckled *vs.* purple sectors | 1 | 537,266 | 537,266** |
| Irradiation dosage rates | 3 | 8,953 | 2,984** |
| Low 2 *vs.* high 2 | 1 | 6,804 | 6,804** |
| 0 *vs.* 1.13r per day | 1 | 205 | 205 n.s. |
| 3.12 *vs.* 6.05r per day | 1 | 1,944 | 1,944** |
| July *vs.* August months | 1 | 11,205 | 11,205** |
| Sectors × dosage | 3 | 8,287 | 2,762** |
| Sectors × months | 1 | 10,185 | 10,185** |
| Error | 6 | 696 | 116 |
| Total (C.F.M.) | 15 | 576,592 | |

** Significant at 1 percent level.
n.s. Not significant at 5 percent level.

are likely to be normally distributed despite the Poisson distribution of individual items, the F test was used.

The trend of speckled-sector means has been presented in simplified form in Figure 1 as two linear regressions. Although the Poisson distribution is not present in these data, the treatment by days interaction was employed as error for the F tests of significance, as was done for the purple-sector data.

The partitioning employed for treatment means in these tests is summarized in the analysis of variance of Table 4. This includes both July and August data separately for both sector types, and in addition tabulates the linear regression coefficients.

Table 5 presents the tests of significance of differences between the July regression coefficients of Figure 1 and Table 4. In these tests it is assumed that differences between regressions as well as deviations from regressions will be normally distributed.

## Major observations

*Spontaneous difference between* V *and* R: Tables 1 and 2 show that a primary difference lies between the mean spontaneous (control) rates for the two types

TABLE 4

*Summary of sector frequency mean squares and regression coefficients from four analyses of variance of irradiation treatment means*

| Source | d.f. | Mean squares | Regression coefficient |
|---|---|---|---|
| July, 1953, speckled sectors | | | |
| Low 5 *vs.* high 4 treatments | 1 | 36,263** | ... |
| Among low 5 treatments | 4 | 1,696** | ... |
| Linear regression | 1 | 5,348** | 1.44 |
| Deviations from regression | 3 | 478 n.s. | ... |
| Among high 4 treatments | 3 | 6,186** | ... |
| Linear regression | 1 | 17,546** | 0.20 |
| Deviations from regression | 2 | 506 n.s. | ... |
| Treatments × days | 48 | 382 | ... |
| July, 1953, purple sectors | | | |
| Linear regression | 1 | 18,776** | 0.14 |
| Deviations from regression | 7 | 24 n.s. | ... |
| Treatments × days | 48 | 44 | ... |
| August, 1953, speckled sectors | | | |
| Linear regression | 1 | 5,010** | 1.94 |
| Deviations from regression | 2 | 5,025** | ... |
| Treatments × days | 18 | 563 | ... |
| August, 1953, purple sectors | | | |
| Linear regression | 1 | 35.39** | 0.16 |
| Deviations from regression | 2 | 1.11 n.s. | ... |
| Treatments × days | 18 | 0.68 | ... |

** Significant at 1 percent level.
n.s. Not significant at 5 percent level.

TABLE 5

*Summary from analyses of variance between July regression coefficients of sector frequency on irradiation dosage*

| Source | d.f. | Error of estimate mean square |
|---|---|---|
| Speckled low (1.44) *vs.* speckled high (0.20) | 1 | 3,967* |
| Deviations from individual regressions | 5 | 489 |
| Speckled low (1.44) *vs.* purple (0.14) | 1 | 4,334** |
| Deviations from individual regressions | 10 | 160 |
| Speckled high (0.20) *vs.* purple (0.14) | 1 | 903* |
| Deviations from individual regressions | 9 | 131 |

* Significant at 5 percent level.
** Significant at 1 percent level.

of sectors: speckled and purple. The Poisson distribution for the items of purple-sector data implies a constant, small probability (of the order of 1/100,000 in controls scored in July) for the occurrence of a sectoring event among the cells sampled within a treatment during a period of a few days. Within this same sample of cells the probability for the occurrence of an event originating a speckled sector is about 100 times larger (of the order of 1/1,000 in controls scored in July).

Tables 1 through 3 also show a significant difference between the two types of sectors in average response over all common irradiation treatments. These spontaneous and average sectoring frequencies provide a phenotypic basis for definition of the *V* gene as mutable and the *R* gene as stable.

*Seasonal effect:* Lower average sectoring frequencies in August than in July are clearly characteristic of the data presented in Tables 1 through 3. This time variable, however, confounds such complex factors as age and development of plants, mineral nutrient depletion, and seasonal environmental changes. The effects of these variables, although potentially separable, cannot be distinguished in the present experiment.

It appears unlikely, however, that temperature differences between months can be held accountable. Both the mean maximum and the mean minimum temperatures for the four weeks previous to each scoring are less than 1°F different between months. Thus despite the known effect of temperature (SAND 1957), mean differences in this variable during floral development cannot account for the observed mean differences in sectoring frequency. Therefore, some additional correlated environmental or metabolic factor is more likely responsible. SPARROW and POND (1956) also have found seasonal differences in somatic mutation rate in snapdragons.

*Season by gene interaction:* Another aspect of the seasonal difference is apparent from Tables 1 and 2 and from the sectors by months interaction of Table 3. The speckled-sector frequency responded by an average decrease from July to August over 30 times that of the purple-sector decrease. Thus, a differential response of the speckled and purple systems in degree of lability to a factor other than chronic gamma irradiation and temperature is inferred.

*Differential response of* V *and* R *at low dose rates:* The response curves for the two genes are very different from each other in slope in the range below about 12 roentgens per day. This is graphically represented in Figure 1, and is shown as well by the sectors by dosage interaction of Table 3 and by the linear regression analyses of Tables 4 and 5. It is suggested that at lower dose rates most speckled-sector increments represent interaction between irradiation and elements of the mutable system which dramatically distinguish it from the more stable somatic system associated with the gene *R*.

*Similar response of* V *and* R *at high dose rates:* Figure 1 and Tables 4 and 5 show that the response curves for the two genes are very similar to each other in slope in the range above about 12 roentgens per day. The primary linear increase in purple-sector frequency within the entire range of radiation rates employed suggests that the mechanism by which the radiation-induced increments are produced may be the same throughout this range. The mechanism could be multiple, with more than one type of radiation-induced event having the same phenotypic consequence: a purple sector. The similarity of response curves suggests that the principal components of the mechanism responsible for both speckled and purple-sector increments may be the same at high dose rates. It is implied that in the higher dosage range most speckled-sector increments represent interaction between irradiation and elements of the mutable system which are in common with the stable system related to *R*.

*Differential response of* V *at low and high dose rates:* Figure 1 and Tables 4 and 5 demonstrate that the slope of the response curve for speckled sectors is greater below about 12 roentgens per day than in the higher range. Since exposure was chronic and for the same period of time, total dose *per plant* is assumed to be proportional to dose rate. A decrease in average sectoring frequency was observed in the later month of August despite the greater accumulated dosage. This suggests that total dose per plant is less pertinent to the sectoring variable than is the dosage administered during the period of development of each flower. While this effective total dose *per flower* is unknown, it also is assumed to be proportional for each treatment to the chronic gamma dose rate applied. Thus Figure 1 may be interpreted as showing response curves for which increasing total doses were administered at increasing intensities. Nonlinearity of the mutable locus curve may indicate that speckled-sector increments are not merely proportional to total dose, but depend as well upon intensity of irradiation.

A greater yield of speckled sectors per roentgen is implied for the low *versus* the high dosage range, and suggests an additional mechanism operative in the low range. It is proposed that this additional mechanism is intimately related to the mutable peculiarity of the genetic system which uniquely determines the spontaneously sectoring phenotype. This system is not only highly labile in more normal environments, but is as well highly responsive in the degree of its lability to relatively small increases in chronic gamma irradiation in a low dosage range. However, this hypersensitivity response saturates near 12

roentgens per day. These results suggest a buffering system operating to stabilize the sectoring mechanism under normal conditions, but sensitive to irradiation such that the protective system is quantitatively reduced by increments of radiation intensity and is effectively eliminated at intensities above 12 roentgens per day.

*Effects transmitted to progeny*

Progeny were grown in 1954 and 1957 from seed produced on members of the clone while growing at the different dose rates. These progeny were cultured under nonirradiation conditions and were classified for their flower phenotypes. A total of 870 individuals in families from the ten irradiation treatments (0–190r per day) were scored in this way.

Data have been analyzed for the percent of speckled progeny and percent of purple progeny (individuals of recessive phenotype) in these families. Five of the families were grown both in 1954 and 1957. In 1957 the frequency of speckled progeny averaged 3.8 percent higher than for families grown from the same seed lots in 1954. Therefore (in view of the known influence of environment on variegation, SAND 1957), 3.8 was added to each of the 1954 averages to adjust to the 1957 level of effect while retaining the differences among the 1954 families. The 1957 data and the adjusted 1954 data were then combined for each family that was grown both years. This was done by means of a weighted average for percent speckled progeny in which the weights were the number of plants observed in the respective year. Since data on purple progeny from the two years did not differ in the average frequency, weighted averages were calculated directly for each family grown both years.

These calculations give values for the percent of speckled progeny and for the percent of purple progeny in ten families coming from one control and nine irradiation treatments of the clone. Results from adjacent treatment levels were pooled as weighted averages to increase the number of plants upon which each frequency is based. These five values for each of the two recessive phenotypes are plotted against weighted average dose rates in Figure 2. The trend for speckled and purple progeny is the same, and the graph shown connects points which represent the average frequency of the two types of recessive progeny. The average frequency (29.7 percent) for the four highest dose rates (2.5, 10.5, 41, and 148r/day) is significantly greater than the average frequency (23.1 percent) for the lowest dose rate (0.25r/day). If individual regressions (without averaging adjacent treatment levels) are calculated for recessive progeny *versus* parental dose rate the linear slopes obtained respectively for speckled, purple and pooled progeny are 0.41, 0.65 and 0.53 in the low dosage range (0–12r/day) in comparison with 0.019, −0.006 and 0.007 in the high dosage range (25–190r/day). Strong trends are evident despite sampling error.

It is clear that chronic gamma irradiation of this heterozygous parental clone (during a period that includes premeiotic stages through development of seed) increases the frequency of recessive individuals in progeny grown under non-

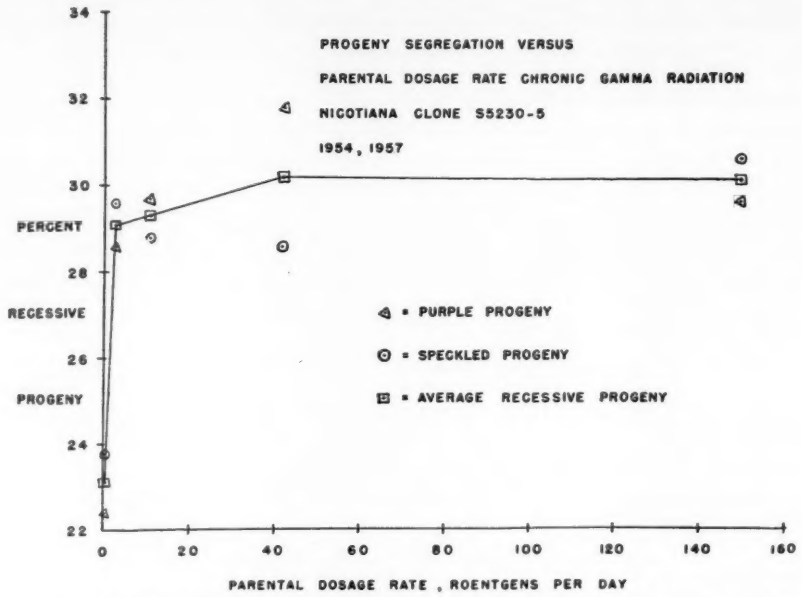


FIGURE 2.—Dosage-response curve showing influence of chronic gamma irradiation of the heterozygous parental clone upon the progeny segregation obtained under non-irradiation conditions. The frequency of recessive progeny responds similarly for the mutable locus *V* and the stable locus *R*.

irradiation conditions. However, the major increment is confined to the low dosage range. Above about ten roentgens per day these data do not show any further significant effect beyond that already produced at this lower dosage rate.

## DISCUSSION

### *Rationale of interpretation*

The favored interpretation, supported by the data, rests upon considerations which are summarized in sequence below and analyzed in later sections of this discussion.

The assumptions made in interpreting the somatic results in terms of an average frequency of mutation per cell division are outlined in a model presenting the view taken of this changing population of cells subject to chronic irradiation.

Reasons are presented for considering the change from low dose rate efficiency to high dose rate inefficiency in producing speckled sectors (Figure 1) as due to an effect of radiation on an *intracellular* system.

Observations are cited which make it unlikely that the progeny segregation results (Figure 2) should be explained in terms of radiation-induced events

after the formation of zygotes on the treated parents. Thus, a *gamete-transmissible* effect is indicated. The somatic and progeny results favor the interpretation that this effect is not solely physiological, but is due to genetic or chromosomal alterations.

In the high dosage range, gene or chromosome deletion is compatible with the observed pattern of results. However, the increase in low dose rate response for progeny segregation (Figure 2) makes it unlikely that deletion is responsible here. If equal deletion of dominant and recessive were assumed, an unchanging progeny segregation would be expected unless $v_s$ were an amorph, which is unlikely in view of its reversible mutability. Thus, a genic alteration or mutation mechanism is favored.

However, it seems improbable that a direct mutation effect should saturate above about 10r/day while adequate genetic targets are present. Therefore, an additional component is postulated in the form of a physiological buffering system against primary mutation of the gene. Mutation then increases with increasing interference by radiation with the buffering system and becomes saturated at a value limited by the gene in the new set of conditions.

Equal potential mutability of $v_{s3}$ and $R$ is indicated (despite their difference in spontaneous instability) by their similar progeny segregation responses to chronic irradiation. It is suggested that this result, as well as differences between somatic and germinal mutability, may be explainable in terms of differential buffering sytsems such as that postulated above.

### *Cell lineage model*

A model is considered in which successive cell divisions during development constitute a cell lineage that generates the mature tissue of the petal epidermis. The number of cells in this mature tissue implies an approximately equal number of cell divisions during its development. Within this developing background tissue a mutation should originate a sector the ultimate size of which will reflect the number of cell divisions in the sector lineage from mutation to maturity. Our analysis has assumed that the total number of mutations under given conditions is proportional to an average mutation rate per dividing cell and to the number of cell divisions. This does not assume mutation to occur only during cell division, but effectively counts a cell of the developing population only once from its origin to division.

If there were a single primordial cell and all of its lineage derivatives were included in the mature petal area scored, then the total number of cell divisions would be one less than the number of mature cells. However, the model cannot be this simple because the standard area scored is square in shape and located near the center of the petal. The mature cells in this area may be substantially fewer than the cell divisions contributing to their lineage. But an estimate is desired of the number of cell divisions leaving *both* daughter cells (or their lineage derivatives) within the limits of the area sampled for mutant sectors at petal maturity. This estimate should exclude divisions of *early* development because

mutations at these stages will result in sectors having their origin points outside the scored area, and such mutant sectors were not counted. The desired estimate also should weight as one half those divisions from which only one of two daughter cell lines is included in the scored area. This desired estimate for the number of cell divisions will be approximated, as for the simpler model, by the number of mature cells in the area sampled.

Expressing the number of mutant sectors as a fraction of the total number of cells in the sampled area should give a minimum estimate of an average mutation rate per cell division. It will be a minimum estimate because divisions are included that occur within mutant sectors unable to show further mutation. Since sectors smaller than eight cells were not counted, an adjustment was made by dividing the number of cells in the scored area by eight to give an effective cell population size. This value was used as an estimate of the number of cell divisions in terms of which the mutation rate was calculated. Average mutation rate, estimated in this way, does not require consideration of the particular cell generation during which a cell division or a mutation occurs.

### *Frequency* versus *timing*

If an experimental treatment alters the mutation rate, there should be a corresponding alteration in the frequency of mutant sectors in the area scored. It is also possible that irradiation could initiate mutation during a cell generation of the sample which would not otherwise be subject to mutation. This could be viewed as an effect of irradiation on the *timing* of mutation or as a *frequency* effect limited to a particular time in development. Our data on sector number will not distinguish such a timing effect from a uniform frequency effect, either of which could result in the same average frequency of mutation. Therefore, interpretation of our results is in terms of an average frequency of mutation per cell division. In this connection, however, it may be noted that CUANY, SPARROW and POND (1958) found indication of no difference in the probability of a cell mutating in flower buds acutely irradiated between 20 and 27 days before opening. At least during this period of flower development a uniform frequency effect is probable.

### *Chronic* versus *acute irradiation*

SAX (1950) found that *Tradescantia paludosa* exposed to chronic gamma radiation showed no further increase in chromosomal aberrations beyond three weeks. This suggests a sensitive three-week period of development of the scored material, with pertinent cell samples entering this phase as survivors relatively unaffected by previous irradiation. The level of effect measured increases with time of exposure until the full critical period of development of the scored material has taken place under irradiation conditions.

This interpretation of a maximum yield as due to a limited period of pertinent chronic exposure is supported by observations of CUANY, SPARROW and POND

(1958) on somatic mutations in Antirrhinum. These authors found: (1) similar frequencies of flower-color sectors on plants scored at the same time after one or four months of chronic irradiation, and (2) reversion to control frequency within a few weeks after removal of plants from the irradiation environment. From comparison with acute irradiation effects, these authors consider that by their technique most sectors counted during chronic irradiation result from mutation which occurred 21–25 days before flower opening.

Several cell generations will be included in this period of time. In our similar Nicotiana material this period is probably about a week followed by about three weeks of development until flower opening.

CUANY, SPARROW and POND (1958) obtained data on the increase in average size of sectors observed on flowers maturing on successive days following acute irradiation. These data permit estimation that the average time interval between cell generations under their conditions is about one day during the period in petal development when events must occur in order to produce mutations scorable by the sampling technique employed. Thus, total dose per cell generation in our material may have the same order of magnitude as the chronic daily dose rate to which these populations of cells were exposed.

For *chronic* irradiation of such a population of dividing cells, the total dose pertinent for comparison with certain effects (including the mutation frequency per cell) of *acute* irradiation may be approximately that administered during the average period of time between cell divisions or cell generations. If this were one day, as estimated above, the pertinent total dose could be numerically equivalent to the chronic daily dose rate. This assumes, in line with the discussion above, that each generation of cells contributing to the scored tissue is composed largely of survivors unaffected by previous irradiation. Thus, the previous irradiation becomes irrelevant. At least it is clear that the pertinent total dose may be considerably less than that administered to the whole plant as chronic irradiation during its development.

### *Low dose rate efficiency*

A sevenfold greater efficiency in production of somatic speckled sectors is suggested by Figure 1 for irradiation in the low dosage range (0–12r/day). Several factors are important in interpreting this result as a radiation effect on an intracellular system.

Although based on a single experiment, it is unlikely (in view of conditions within that experiment) to be a spurious effect. In addition to its statistical significance (Tables 4 and 5), it was obtained in two independent periods of somatic scoring of the experimental clone, and in segregations of progeny from this irradiated clone. Its appearance in progeny tests indicates also that the pattern of the somatic results is not merely a consequence of somatic scoring technique.

Viewed as a departure from linearity, the pattern seems to be a one-directional change in slope, rather than a rise-fall-rise as for instances of nonlinear response in snapdragons reported by SPARROW and POND (1956). In addition the magnitude

of our 0–10 roentgens per day response represents 30 percent of the 0–200 roentgens per day response, in comparison with a corresponding 10 percent proportion shown in the snapdragon material. SPARROW usually, as well as SMITH (1958), has found linear somatic sector response at low dose rates for stable flower color genes. This is in agreement with our results showing linear response of purple sectors involving the stable gene *R*. Indeed, the *R* system response serves as an internal control in our material: the nonlinear speckled-sector response involving the unstable gene *V* was obtained in the same petal tissue giving linear *R* response. Such a result makes it less likely that either differential cell selection or an external environmental gradient is the cause of the greater low dosage response of *V*. Also, the control speckled-sector frequency is colinear with the low dosage response. This would be unexpected by a field gradient hypothesis because the control plants were grown in a field spacially distant and discontinuous from the irradiation field. Thus the favored interpretation is that the nonlinearity represents interaction between irradiation and the biological system.

On this basis the slope differences of Figures 1 and 2 imply corresponding differences in relative target size or system sensitivity as judged from recoverable products. Since the biological target is a population of developing cells, slope changes could result either (1) from change in size of the target population or (2) changes in one or more critical intracellular components. An effect of increasing sector frequency with increasing dose rate is to produce a higher proportion of mutant cells in the final population, as an integration of events occurring during a number of cell generations. A consequence is a decreasing net target population of nonmutant cells, and an expected saturation effect. However, by this mechanism saturation should not occur with so large a residual target population: in somatic tissue 75–95 percent of the cells remain nonmutant, and in the progeny tests a minimum of about 70 percent of the individuals are nonmutant. Also the effect should be observed in somatic tissue for purple as well as speckled sectors, but is not. Thus it is inferred that a change in an intracellular target system, affecting the frequency of detected events per roentgen, probably accounts for the observed nonlinearity.

### *Prezygotic timing*

Since chronic irradiation was maintained from pregerminal through germinal stages to postzygotic development, no direct measure is available for the time of occurrence of events affecting progeny segregation. However, relative uniformity in the frequency of recessive progeny at high dosages suggests that no large contribution is made by events at the zygotic stage of development, but that the major detected component is prezygotic in its timing. It would be expected that increasing frequency of zygotic mutation or deletion or physiological effects would increase the frequency of recessive progeny and of plants grossly chimeral for recessive tissue, in accordance with the somatic results for petals. No increase in the frequency of grossly chimeral progeny was observed with increasing dose rate. SINGLETON (1954) in results from chronic irradiation of maize also noted

no apparent increase in the percent of fractionally mutant kernels with increased radiation. The favored interpretation is that radiation-induced events after the formation of zygotes on the treated Nicotiana plants are not an important component in the detected pattern of segregation results.

*Genic effect*

The progeny segregation results are considered generally incompatible with a view that the radiation effects are solely at a functionally physiological or cytoplasmic level rather than at a genic, chromosomal or nuclear level. Such a model would require the postulate of a persistent physiological change transmissible through a sexual generation and effectively inactivating a gene. The hereditary persistence of the change in a character which is known to be primarily under genic control argues for a genic or chromosomal alteration. In addition, the somatic results show discrete sectors of qualitatively similar tissue. Gradations in quality of effect would be considered more likely if the critical target were solely a cytoplasmic multiply-particulate or disperse physiological system. Ultimate effects upon a more localized control center such as the gene appear more tenable. Thus, while physiological effects are not excluded, it is considered that such effects must subsequently result in genic alterations to conform with the observed somatic and progeny results.

*Intracellular target in high dosage range*

Heterozygous individuals were employed by PATTERSON (1929) to detect X-ray-induced somatic modifications in Drosophila, which were explained in terms of gene mutation and deletion. A known part of the intracellular target in our material is the genetic system heterozygous for speckling ($v_{s3}/v_s$) and for purple color ($R/r$). Limiting attention to the high dosage range, a gene deletion mechanism is consistent with (1) the similar somatic response for speckled and purple sectors, and (2) the apparent absence of continued change in progeny segregation response.

Mechanical deletion could be impartial to unstable and stable genes, and to dominant and recessive alleles. In somatic tissue, deletion of either dominant could result in a visible hemizygous recessive sector, whereas deletion of either recessive would be visibly undetectable. Pregerminal or germinal deletion, even if its frequency were to increase, should leave the proportion of dominant to recessive alleles unaltered. If gametes bearing deletions are relatively nonfunctional a constant progeny segregation is expected, and this is observed at the higher dose rates. Thus, as a major mechanism, gene deletion or inactivation is in agreement with the pattern of results in the high dosage range.

*Intracellular target in low dosage range*

Such a deletion mechanism is assumed to operate in the low dosage range as well. However, the greater speckled-sector slope in the low dosage range (Figure 1) implies a sevenfold greater target size or more sensitive target quality. The

similar increase in low dosage response for progeny segregation (Figure 2) makes it unlikely that gene deletion is responsible. An additional mechanism seems required. Somatic tissue disorganization (GOODSPEED 1929) is inadequate in this case. As discussed above, the hereditary persistence of the effect and the discreteness of the qualitatively similar somatic sectors argue for genic alteration or mutation.

But the hypersensitivity disappears at dose rates above about ten roentgens per day. It seems improbable that a direct mutation effect should saturate at this dose rate while adequate genetic targets are present, as is implied by the remaining populations of nonmutant cells and progeny. Adequate interpretation appears to require an additional component which is effectively eliminated by the irradiation dosage at which the saturation effect is observed. From background radiation levels to this critical dose rate this component appears to be quantitatively reduced in the efficiency with which it interacts with the gene.

Insight into a possible function for such a system is aided by the following approach. If the high dosage rate response of the mutable locus (Figure 1) is extrapolated to zero dosage rate the sector frequency predicted is about 15 sectors greater than the actually observed control frequency. It is suggested that the control frequency is lower than this expectation because of the presense of a system buffering against primary mutation of the gene. In the absence of this homeostatic system spontaneous changes in the gene $v_{83}$ would be even more frequent than their already dramatically high rate. Such a buffering system could be specific or general in its significance and could owe its initiation to either genetic or cytoplasmic elements.

If we are dealing here with interaction between irradiation and a buffering system against mutation, detection of the mutational consequences may depend upon employment of chronic irradiation. Recovery from acute irradiation damage to the buffer system could prevent the realization of mutational consequences, particularly for a component of mutation more likely to occur at some limited time in cell development or depending upon a process requiring an extended period of time. An appropriate time or process could be that of gene duplication for which a possible buffer system could be one maintaining an adequate substrate pool. Regardless, the observed low chronic dose rate at which saturation occurs suggests a physiological target, such as a biochemical system's rate of synthesis or quantity of product, having subsequent significance for the gene. Thus the present interpretation involves a physiological system having a quantitative buffering effect against gene mutation and being affected quantitatively by chronic gamma irradiation. Mutation then increases with increasing interference by radiation with the buffering system and becomes saturated at a value limited by the gene in the new set of conditions.

Pertinent to the plausibility of inactivation of a suppressor of the sectoring system is the work of GLASS (1944) in which a 1000r treatment of ten-hour Drosophila embryos gave 95 percent erupt mutant individuals. Specific inactivation of a suppressor gene of erupt (present in the stock) was considered an effect

of X-rays acting at the physiological level on the suppressor system rather than directly on the gene or chromosome. Pertinent also is such work as that of MOORE and MCDONALD (1955) in which X-ray inactivation of a specific enzyme solution was shown to be an exponential function of radiation dose. Other work, for example that of WOODWARD and CLARK (1955) with Neurospora, indicates that a biological process may be stimulated by X-ray treatment, followed by disappearance of the stimulus at higher dosages. In addition NEWCOMBE and MCGREGOR (1954) have studied saturation effects shown by radiation induced mutation in Streptomyces. We feel confident for our material in (1) excluding an intercellular selection effect and (2) favoring an intracellular response relatively specific to the mutable gene, because the saturation effect was not observed for the stable gene in the same samples of somatic tissue.

It should be emphasized that the detectable mutation in our irradiated material is viewed as a relatively specific change from $v_{s3}$ to $v_s$, a change which also occurs spontaneously, which is subject to temperature influence on its frequency, and which is considered potentially reversible (SAND 1957). Thus the hypothesized buffering system may interact with mutation over only a specific range of a larger mutational spectrum, and may be sensitive to continuous temperature levels as well as to continuous irradiation levels. This interpretation suggests, in addition, that both mutational yields correlated respectively with a chronic temperature increment and with a chronic irradiation increment in the low dosage range may represent mutational events identical to those occurring spontaneously in this plant material.

Exponential increase in somatic mutation was indicated for one variety of carnation subjected to chronic gamma irradiation by RICHTER and SINGLETON (1955). A similar greater than linear increase with dose rate was found by SINGLETON (1954) for gametic mutations scored for endosperm characters in maize. In addition, recent results of RUSSELL, RUSSELL and KELLY (1958) indicate dependence on dose rate for radiation induced mutation in mice. The above observations show greater mutational yield at high radiation intensity, while our results indicate relatively greater yield per roentgen at low intensity. However, the biological significance could be very similar. RUSSELL's model suggests a "protective" or "repair" system damaged only at high radiation intensities, whereas our model from different material suggests a "protective" system sensitive to damage even at low radiation intensities.

By a different approach the changes from $v_{s3}$ to $v_s$ in this heterozygous clone could be viewed as limited somatic gene conversions (HAGEMANN 1958). The postulated buffering system could then be described as an inhibitor of gene conversion.

### *Mutable* versus *stable gene*

It is tempting to speculate concerning the possible significance of these results in relation to the differential spontaneous instabilities of these genes at the *V* and *R* loci. As shown in Tables 1 and 2, spontaneous speckled-sectors (attributed

to mutation from $v_{s3}$ to $v_s$) are more than 100 times more frequent than purple sectors (attributed to mutation from $R$ to $r$). Despite this difference in spontaneous instability, the similar progeny segregation responses to chronic irradiation (Figure 2) suggest equal potential mutability of $v_{s3}$ and $R$ under conditions that remove secondary stabilizing influences. It may be indicated that the high spontaneous mutability of the $V$ system is not inherent in the gene $v_{s3}$ itself, but arises from the presence of inadequate homeostatic forces external to this gene. In terms of a buffer system against mutation this homeostatic force could be considered inefficient in protecting against errors in gene duplication.

This analysis is in accord with the interpretation of DEMEREC (1955). By this view gene stability may result from the presence in the gene's immediate environment of all the components necessary for exact self-duplication, rather than solely from inherent stability of chemical structure itself.

### *Somatic* versus *germinal stability*

Meiotic chromosomes of Tradescantia are more radiation sensitive than those in somatic nuclei (SPARROW and POND 1956) as judged from relative frequencies of cytological aberrations. SINGLETON (1954) and DOLLINGER (1954) from chronic irradiation in maize found premeiotic mutations to be only rarely detected. These results indicate meiotic or gametic timing of most detected events, and suggest with other work that the sensitivity of germinal tissue to radiation-induced mutation is greater than the sensivitity of somatic tissue.

The greater somatic stability of the $R$ system in our Nicotiana material, both spontaneously and under chronic irradiation, may indicate additional somatic buffering for $R$ sufficiently specific not to function in the same cell for $V$. A similar difference in a more general buffering system between somatic and germinal tissue could account for differential sensitivity of these tissues to radiation-induced mutation. The difference between $V$ and $R$ in somatic response parallels the difference between germinal and somatic response for $R$. This suggests a corresponding parallel difference, perhaps in buffering systems against mutation, to account for (1) differences between germinal and somatic mutability and (2) differences between mutable and stable gene sensitivity.

### SUMMARY

Data are presented for somatic and germinal effects of chronic gamma irradiation on a mutable allele of the $V$ gene and on the stable $R$ gene both of which affect flower color in a clone of Nicotiana. Growing plants of the heterozygous clone ($v_{s3}/v_s$, $R/r$) were subjected to different levels of chronic gamma irradiation. The somatic effect on a mutable gene system was compared with a stable gene system in the same tissue by counting speckled sectors (changes from $v_{s3}$ to $v_s$ phenotype) and purple sectors (changes from $R$ to $r$ phenotype) on irradiated and control plants. Similarly, germinal effects on the same gene systems were studied by counting speckled and purple individuals in progeny from the irradiated and control plants.

In unirradiated controls spontaneous speckled sectors involving the mutable gene are about 100 times more frequent than purple sectors. Lower average sectoring frequencies were observed in August than in July from the same plants, but the seasonal difference for speckled sectors was 30 times greater than that for purple sectors.

The somatic response curve for the mutable gene is about ten times greater in slope than the purple sector response in the treatment range below about 12 roentgens per day. However, the somatic response curves for the two genes are very similar to each other in slope in the range from about 12–190 roentgens per day. The somatic response curve for speckled sectors is nonlinear, showing a sevenfold greater slope below 12 roentgens per day than in the higher range.

The germinal response curves are similar for the mutable and stable genes. Chronic gamma irradiation of this heterozygous parental clone increases the frequency of recessive individuals in progeny grown under nonirradiation conditions. The major increment is confined to the low dosage range. Above about ten roentgens per day these data show no further significant effect on progeny segregation beyond that already produced at this lower dosage rate.

A cell lineage model is described for relating sector frequencies and mature cell populations in terms of average somatic mutation rates per cell division.

Irradiation dose per somatic cell generation is estimated to have the same order of magnitude as the chronic daily dose rate to which these populations of cells were exposed.

The results indicate prezygotic timing of most events affecting the progeny segregations.

Nonlinear mutational response indicating greater efficiency of low dose rates is interpreted as due to change, brought about by irradiation, in an intracellular target system.

As a major mechanism for response increments in the high dosage range, gene deletion or inactivation is tenable. However, in the low dosage range the somatic and progeny results appear to require an increasing component of effect including genic alteration or mutation. In addition, low chronic dosage rate saturation in the presence of adequate genetic targets suggests a physiological system, buffering against mutation, and quantitatively sensitive to damage by low dose rates of irradiation.

The similar progeny segregation responses to chronic irradiation indicate equal potential mutability of $v_{s_2}$ and $R$ under conditions that remove secondary stabilizing influences. The greater somatic stability of the $R$ system suggests additional somatic buffering for $R$ sufficiently specific not to function for $V$.

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# RELATIONSHIP BETWEEN THE INTRACELLULAR LOCALIZATION OF BETA-RADIOISOTOPES AND THEIR MUTAGENIC EFFECT

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IN the course of studies on the mutagenic effect of radioactive isotopes on microorganisms, the author has observed that the mutagenic effect of radioactive sulfur ($S^{35}$) is much less than that of radioactive phosphorus ($P^{32}$). Further observations have suggested that this difference between mutagenic effects of two isotopes producing beta-rays is derived not from the difference of energy power of the isotopes but from the intracellular localization of the isotopes. This consideration has been raised by the observation that the mutagenic effect of radioactive sulfur is much less than that of radioactive phosphorus under the condition where the radioactive energy of the former per mg of cells is larger than that of the latter per mg of cells.

## MATERIALS AND METHODS

*Strain: Mycobacterium avium*, strain Jucho (an avirulent strain), was used throughout the study.

*Irradiation:* The organism was inoculated into liquid media containing radioisotopes and cultured to form a pellicle. The culture was continuously irradiated by the isotope during the growth as described by RUBIN (1954), and the isotope was taken into the cells.

*Media:* Modified Sauton medium used in the study consisted of 50 ml of glycerin, 2.0 gm of sodium citrate, 0.5 gm of magnesium sulfate, 0.5 gm of dibasic potassium phosphate, 0.05 gm of ferrous sulfate, 4.0 gm of sodium glutamate, and 950 ml of distilled water. The pH was adjusted to 7.2 with ten percent sodium hydroxide. The sulfur-deficient medium consisted of 50 ml of glycerin, 2.0 gm of sodium citrate, 0.5 gm of magnesium chloride, 0.05 gm of ferric ammonium citrate, 4.0 gm of sodium glutamate, and 950 ml of 0.033 M phosphate buffer (pH 7.1). These media were dispensed in 50 ml amounts in 200 ml Erlenmeyer flasks and autoclaved at 115°C for 30 minutes. The media were inoculated by a small inoculum consisting of approximately 1,000 viable cells and incubated at 37°C. Modified Sauton medium containing 0.5 microcuries of $P^{32}$-orthophosphate (Radiochemical Center, Amersham, England) per ml of medium and sulfur-deficient medium containing ten microcuries of $S^{35}$-sulfate (Commissariat a l'energiew atomique, Saclay, France) per ml of medium were used for the continuous irradiation.

In order to increase the incorporation of $S^{35}$ into cells, the organism was subcultured three times in sulfur-deficient medium containing no radioisotope and thereafter inoculated to sulfur-deficient medium containing $S^{35}$-sodium sulfate

as the sole sulfur source. Transfers in sulfur-deficient media slightly affected the growth, but the mutation frequency was not affected by the transfers.

$P^{32}$-phosphate was added to modified Sauton medium containing 0.05 percent dibasic potassium phosphate as a component of the medium. This decreased the incorporation of $P^{32}$ into bacterial cells.

*Determination of the numbers of streptomycin-resistant mutants and isoniazid-resistant mutants in the total viable population:* Ratios of streptomycin-resistant mutants and isoniazid-resistant mutants, arising spontaneously or induced by the radioisotopes, occurring in the total viable population were used as markers of the mutation frequencies. Counts were performed in the conventional manner by utilizing Sauton agar plates. Sauton agar medium consisted of modified Sauton medium added to three percent agar. One tenth milliliter of each of the different dilutions of cell suspension, prepared by homogenizing cultures with glass beads and suspending them in saline, was inoculated with a roller onto each of five plates, 9 cm in diameter: containing no drug, containing 1 $\mu$g/ml or 10 $\mu$g/ml of streptomycin, and containing 10 $\mu$g/ml of isoniazid. Plates were incubated at 37°C for five days, and the number of colonies was then counted in the usual manner. Sterile dihydrostreptomycin sulfate (manufactured by Meiji Co.) and isoniazid (manufactured by Tanabe Co.) were added to Sauton agar media immediately before use. Isoniazid was sterilized by Seitz filtration of solutions.

*Measurement of radioactivity:* Radioactivity of supernatants of cultures, of distilled water used for washing cells, and of bacterial cells was measured as follows: Bacterial cells (bacillary mass) of each flask were collected by centrifugation at 3,000 r.p.m. for 15 minutes. The cells were washed repeatedly with 40 ml of distilled water until the washing-water had a radioactivity of less than about 1.5 times the background radioactivity. The resulting cell mass was weighed while in the centrifugation tube, and total growth per flask was determined by subtracting the weight of the tube. The cells and glass beads were shaken together and the cells were suspended in distilled water. The optical density of the cell suspension was measured by a Leitz's Rouy-Photometer; the number of milligrams, wet weight, of cells per milliliter of suspension was determined by comparing readings with a previously prepared standard curve. This number was used to determine the radioactive "counts per minute (c.p.m.)" per mg, wet weight, of cells.

Each 0.2 ml of supernatants, washing-waters, and cell suspensions was placed in an aluminum dish and dried. The radioactivity of these materials was counted by a Geiger-Mueller counter (Electronic Counter, GM481D, Philips, Holland). The appropriate corrections for background and natural decay were applied.

## RESULTS

### *Radioisotope uptake by bacterial cells*

Amounts of uptake of radioisotopes by bacterial cells (incorporation and adsorption into cells) determined by the method described above are shown in Table 1. Uptake of $P^{32}$ remained relatively small, probably due to competition

TABLE 1

*Comparison of radioactivities of $P^{32}$ and $S^{35}$ taken up by bacterial cells grown in modified Sauton medium containing 0.5 microcuries of $P^{32}$-phosphate per ml (after three days of incubation) and in sulfur-deficient medium containing ten microcuries of $S^{35}$-sulfate per ml (after four days of incubation)*

| Radioisotope added to medium | Radioactivity of cells (wet weight) per mg | Total growth per flask (wet weight) | Ratio of uptake by bacterial cells* |
|---|---|---|---|
| $P^{32}$: 0.5 microcuries per ml × 50 ml = 25 microcuries per flask | 902 c.p.m.† (0.0085 microcuries per mg (wet weight) of cells) | 400 mg | 13.6 percent (360,000 c.p.m. per 2,650,000 c.p.m. |
| $S^{35}$: 10 microcuries per ml × 50 ml = 500 microcuries per flask | 13,609 c.p.m.† (0.618 microcuries per mg (wet weight) of cells) | 600 mg | 81.6 percent (8,160,000 c.p.m. per 11,050,000 c.p.m. |

* Ratio of the amount of radioactivity taken up by bacterial cells per total amount of radioactivities found in the supernatant, water used for washing, and washed bacterial cells.
† Counts per minute.

with nonradioactive phosphate coexisting in the medium. The amount of $P^{32}$ taken up by the bacterial cells was 13.6 percent of the total amount of $P^{32}$ added to the medium. Uptake of $S^{35}$ was relatively large, probably because the radioactive sulfate was the sole sulfur source and the organism had grown in sulfur-deficient medium. The amount of $S^{35}$ taken up by the cells was 81.6 percent of the total amount of $S^{35}$ added to the medium.

Radioactivity of cells grown in medium containing $P^{32}$ was 902 c.p.m per mg, wet weight, of cells, which corresponds to about 0.0085 microcuries per mg, wet weight, of cells. On the other hand, radioactivity of cells grown in medium containing $S^{35}$ was 13,609 c.p.m. per mg, wet weight, of cells, corresponding to about 0.618 microcuries per mg, wet weight, of cells.

### *Mutation frequencies in bacterial cells*

Mutation frequencies were determined by utilizing streptomycin-resistant mutants and isoniazid-resistant mutants as markers. The determination was performed after three to four days of incubation when it appeared that the growth had reached the end of the exponential phase.

As shown in Table 2, the uptake of $P^{32}$ significantly increased the mutation frequencies; more than a tenfold increase of mutation was noted. The uptake of $S^{35}$ did not increase the mutation frequencies in spite of the fact that the radioactivity of $S^{35}$ taken up by the cells and that added to the medium was larger than that of $P^{32}$.

Total growth and viability of the organisms were reduced to some extent by the uptake of $P^{32}$, whereas they were not significantly affected by the uptake of $S^{35}$ (Table 2).

TABLE 2

*Frequencies of spontaneous and induced mutations in cultures grown in modified Sauton media with and without 0.5 microcuries of $P^{32}$-phosphate per ml and in cultures grown in sulfur-deficient media with and without ten microcuries of $S^{35}$-sulfate per ml and with 100 μg of sodium sulfate per ml of medium*

| | Modified Sauton medium* | | Sulfur-deficient medium† | | |
|---|---|---|---|---|---|
| Mutation frequency | Control | With $P^{32}$‡ | Control | With $S^{35}$§ | With Sulfate‖ |
| Mutants resistant to 1 μg/ml of streptomycin per $10^5$ viable cells | 1.08 | 43.2 | 0.972 | 0.500<br>4.30 | 1.28 |
| Mutants resistant to 10 μg/ml of streptomycin per $10^8$ viable cells | 10.2 | 115.0 | 4.14 | 15.0<br>31.5 | 4.77 |
| Mutants resistant to 10 μg/ml of isoniazid per $10^5$ viable cells | 6.52 | 98.5 | 3.42 | 11.0<br>14.9 | 8.07 |
| Total growth per flask | 880 mg | 400 mg | 330 mg | 600 mg | 602 mg |
| No. of viable cells per mg (wet weight) | $4.88 \times 10^6$/mg | $1.25 \times 10^5$/mg | $1.00 \times 10^6$/mg | $1.78 \times 10^6$/mg | $1.29 \times 10^6$/mg |

* Determined after three days of incubation.
† Determined after four days of incubation.
‡ Added with 0.5 microcuries per ml of $P^{32}$-phosphate.
§ Added with 10 microcuries per ml of $S^{35}$-sulfate.
‖ Added with 100 μg. per ml of $Na_2SO_4$–10 $H_2O$.

The possibility that the increase in mutation frequencies was actually a selective mechanism could be excluded, since both streptomycin-resistant and isoniazid-resistant mutants grew as well as did sensitive organisms in the presence or absence of radioisotopes, and the mutation frequencies were not affected by the medium utilized (This can, in part, be seen in Table 2.).

## DISCUSSION

Both $P^{32}$ and $S^{35}$ produce beta-rays. Under the conditions used in the test, the amount of radioactivity of $S^{35}$ added to the medium and the uptake of $S^{35}$ by bacterial cells were greater than those of $P^{32}$. The count for $S^{35}$ was about fifteenfold larger than that for $P^{32}$ when measured by c.p.m. per mg, wet weight, of cells. Nevertheless, the mutagenic effect of $S^{35}$ under these conditions was much less (almost none) than the mutagenic effect of $P^{32}$. This discrepancy between the radioactivity and the mutagenic effect, that is, the fact that the mutagenic effect is not parallel with the amount of radioactivity could be interpreted as follows: it is obvious that $P^{32}$ incorporates into the desoxyribonucleic acid (DNA), genetic materials, whereas $S^{35}$ does not incorporate into the DNA.

STENT and FUERST (1955) and FUERST and STENT (1956) reported on the inactivation of bacteriophage and *E. coli* by decay of incorporated $P^{32}$. KAUDEWITZ, VIELMETTER, and FRIEDRICH-FREKSA (1958) reported on the mutagenic effect on *E. coli* associated with the decay of $P^{32}$. If mutations, as reported by these authors, are produced by decay of the incorporated isotope, it would be expected that $P^{32}$, which can incorporate into the DNA, would be able to cause mutations and $S^{35}$, which cannot incorporate into the DNA, would be unable to do so. Thus, the high mutagenic efficiency of $P^{32}$ may be due to the fact that $P^{32}$ decays in the DNA, and the fact that $S^{35}$ has no significant mutagenic effect may be related to the lack of incorporation of $S^{35}$ into DNA. Under these conditions, it appears that intracellular radioactive substances do not always increase the mutation frequency and, at least in the case of beta-isotopes, intracellular radioactive substances existing outside the DNA do not have significant mutagenic effect, even when they exist intracellularly. The results obtained in the present study suggest that the mutagenic effect of radioisotopes is significantly affected by the intracellular localization of radioisotopes.

It is also of interest that the viability of the organism was damaged by the uptake of $P^{32}$ but not by that of $S^{35}$. This suggests that intracellular irradiation of genetic materials, from outside the DNA, does not significantly cause the viability damage but that incorporation of radioactive substances into DNA may cause such damage.

STRAUSS *et al.* (1956) reported on the lethal effect of incorporated $P^{32}$ and $S^{35}$ in *Neurospora crassa*. While the lethal effect of $S^{35}$ has not been demonstrated in the present study, it may be that incorporated $S^{35}$ exhibits a lethal effect derived from its decay in different sites (outside the DNA) of cells. The fact that no significant lethal effect of $S^{35}$ was found in the present study may be due to the relatively short period of observation.

## SUMMARY

Mutation frequencies were determined in *Mycobacterium avium* grown on modified Sauton media with and without 0.5 microcuries of $P^{32}$-phosphate per ml, and on the organism grown on sulfur-deficient media with and without 10 microcuries of $S^{35}$-sulfate per ml of medium. An experimental condition was produced, under which the uptake of radioactive sulfur by bacterial cells was somewhat larger than the uptake of radioactive phosphorus. Under these conditions, a more than tenfold increase of mutation frequency occurred in cells irradiated by radioactive phosphorus, whereas no significant increase of mutation frequency occurred in cells irradiated by radioactive sulfur.

The results have indicated that radioactive phosphorus, which can incorporate into the DNA, can exhibit a significant mutagenic effect, while radioactive sulfur, which cannot incorporate into the DNA, can exhibit no significant mutagenic effect. It appears conceivable that the mutagenic effect of radioactive isotopes producing beta-rays is not always parallel with the radioactivity but may be affected by the intracellular localization of the isotope.

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# HETEROZYGOTE VIABILITY OF A SECOND CHROMOSOME RECESSIVE LETHAL IN DROSOPHILA MELANOGASTER[1]

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THE present study is concerned with the second chromosome recessive lethal *l(2)55i* in *Drosophila melanogaster*. This gene has been studied previously by MUKAI and BURDICK (1959) who showed that single gene heterosis is associated with this particular lethal. In the previous work, four experimental populations, differing with respect to initial lethal gene frequency and genetic background, were established. In order to estimate the viability of the lethal heterozygotes compared to the wild type homozygotes, these populations were sampled for several generations and the frequency of lethal heterozygotes in each generation determined. These frequencies were then compared to the frequencies which would have been expected if the lethal heterozygote had shown no selective advantage over the wild type. The frequency of lethal heterozygotes in the experimental populations reached an equilibrium point of about 0.42.

Various factors relating to the maintenance of *l(2)55i* in populations will be considered in this paper. How is the heterotic effect expressed? Do lethal heterozygous females lay more eggs than the wild type females? Do lethal heterozygous males in competition with wild type males have an advantage in mating ability? Do lethal-carrying sperm have a competitive advantage over sperm carrying the wild type allele? Is zygotic viability of lethal heterozygotes higher than that of wild types? What is the relative importance, or what is the contribution, of each of these four factors to the maintenance of *l(2)55i* at such a high frequency?

Other factors of interest concerning the gene *l(2)55i* are presented including the present estimate of the lethal heterozygote frequency in the stock from which the gene was originally extracted, present estimate of the frequency of lethal heterozygotes in the four experimental populations, and time of lethality of the gene in homozygous condition.

## MATERIALS

The following stocks were developed by BURDICK and his collaborators:

W-1 is a wild type stock collected at Erie, Pa. in the fall of 1954 and maintained by random mating.

W-11 is a wild type stock inbred from A. E. Bell's stock, CP.

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W-109 is a homozygous wild-type stock extracted by the CMI technique (BURDICK 1954a) from W-11.

C-101 has the second chromosome lethal *l(2)55i* balanced over *Ins(2)SM1*, *al*$^2$ *Cy sp*$^2$ with first, third and fourth chromosomes homozygous. This stock and W-101 were extracted from a single W-1 female by the CMI technique. W-101 is a completely homozygous stock. Both C-101 and W-101 have been maintained by random mating.

The following populations were established by MUKAI:

Population 1 was established by 50 pairs of lethal heterozygotes (obtained as virgin offspring from a cross between C-101 and W-101) in each of seven half-pint bottles. The genetic background of the lethal heterozygotes in this population is that of a single W-1 female.

Population 2 was established in the same way as Population 1 except that the lethal heterozygotes were obtained from a cross between C-101 and W-109. The genetic background for the lethal heterozygotes in Population 2 is 50 percent W-1 and 50 percent W-109.

Population 1(3–4) has no second chromosome lethal and was established from a single pair mating of lethal-free flies in the 22nd generation of Population 1. Therefore, the genetic background of this population is that of Population 1. This stock is maintained by random mating.

Population 3 consists of eight bottles. At its origination, 50 pairs of lethal-free flies from Population 1(3–4) and lethal heterozygous flies from Population 1 were put into each bottle. The expected frequency of lethal heterozygotes in the original 100 flies was 0.05. The genetic background of this population is that of Population 1.

Population 4 consists of ten bottles and was established in the same manner as Population 3 except that the lethal-free flies came from Population 2(1–4) (established by a single pair mating of lethal-free flies in the 17th generation of Population 2) and the lethal heterozygotes came from Population 2. The expected frequency of the lethal heterozygote in the starting generation of Population 4 was 0.05. The genetic background of this population is that of Population 2.

Populations 1, 2, 3 and 4 were maintained by MUKAI for the first 15 generations in the following manner: A random sample of 50 pairs of flies from each bottle was transferred to a new bottle every 15 days. In order to avoid intergeneration mating, all flies were removed from the bottles nine days after transfer. After 15 generations, each bottle of each population was maintained by mass transfer every 15 days with no precautions taken to avoid intergeneration matings.

All stocks were maintained and all experiments carried out at a temperature of 25 ± 1°C. The culture medium was that described by BURDICK (1954b). Active dry yeast was added to the bottles at the time of use.

### *Time of action of homozygous* l(2)55i

The following procedure was used to determine the time of action of homo-

zygous *l(2)55i*. A cross between Population 1(3–4) and C-101 yields two types of offspring, as follows:

$$+/+ \times Cy/l \longrightarrow +/l \text{ and } +/Cy$$

Ten pairs of $+/l$ flies resulting from such a cross were randomly selected and put into a bottle. Ten pairs of $+/+$ flies from Population 1(3–4) were placed in another bottle for use as a control. When the flies had been in the bottles for 24 hours, each group of 20 flies was transferred to an egg-laying chamber similar to that described by KING (1955). The two egg-laying chambers were placed on medium in Petri dishes. Charcoal was added to the medium in order to facilitate egg counting. Immediately before placing the egg-laying chamber on the food, the surface of the food was seeded with a solution of acetic acid, water and active yeast.

Flies were allowed to deposit eggs on the medium for approximately 12 hours. After 12 hours the egg-laying chambers were transferred to new medium. This procedure was repeated until four groups of eggs were obtained from each of the two types of matings. A total of 1,597 eggs was included in the experiment.

The following counts were made for each of the eight groups of eggs: (1) number of eggs deposited, (2) number of eggs which hatched into larvae, (3) number of larvae which pupated, and (4) number of pupae which developed into adult flies.

One fourth of the zygotes from the mating $+/l \times +/l$ were expected to be homozygous for the lethal gene and, therefore, were expected to die at some time during development.

The analysis of variance procedure was applied to the larvae/egg, pupae/larvae and imago/pupae ratios. In no case was there a significant difference between groups of eggs within mating types. There was a highly significant difference between mating types in the pupae/larvae ratios, but no difference between mating types in the larvae/egg or imago/pupae ratios. The analysis for pupae/larvae ratios is given in Table 1. It was assumed that there was no interaction between groups and mating types.

The four groups of each mating type were pooled in order to estimate the relative pupation rate of offspring of the cross $+/l \times +/l$. The results are shown in Table 2.

TABLE 1

*Analysis of variance in pupae/larvae ratios*

| Source | d.f. | Mean square | F |
|---|---|---|---|
| Among groups | 3 | 23.7087 | 2.5289 |
| Between mating types | 1 | 1002.3040 | 106.9147** |
| Error | 3 | 9.3748 | |
| Total | 7 | | |

** Significant at the 0.01 level.

TABLE 2

*Numbers of larvae and pupae produced from two types of matings in the test for time of lethality*

| | Type of mating | |
|---|---|---|
| | $+/l \times +/l$ | $+/+ \times +/+$ |
| Larvae | 678 | 504 |
| Pupae | 481 | 469 |
| Pupae/larvae ratio | 0.7094 | 0.9306 |
| Relative pupation rate = 0.7094/0.9306 = 0.7623 | | |

If this lethal produced its effects solely in the larval stage, a relative pupation rate of 0.75 would be expected. The relative pupation rate of 0.7623 is sufficiently near 0.75 to conclude that homozygous *l(2)55i* acts during the larval period by preventing larvae from making the physiological changes necessary for development into the pupae stage.

### *Frequency of* l(2)55i *heterozygotes in W-1*

The gene *l(2)55i* was first found in, and extracted from, the W-1 (Erie) wild type stock. At that time the frequency of the lethal heterozygotes was estimated at 0.34 (BURDICK and MUKAI 1956). This stock was retested to determine the frequency of the lethal at the present time.

Males were randomly selected from the W-1 population and mated individually to C-101 females. These matings were one of the following two types:

1. $Cy/l \times +/+ \longrightarrow$ 50 percent Curly and 50 percent wild type.
2. $Cy/l \times +/l \longrightarrow$ 67 percent Curly and 33 percent wild type.

Therefore, if in the offspring of these single male matings a ratio of 1 Curly:1 wild type was found, the male parent was scored as not carrying the lethal gene; if the ratio was 2 Curly:1 wild type, the male parent was scored as a lethal heterozygote. In those cases where there were insufficient offspring and/or a $\chi^2$ discrimination could not be made between the two ratios, the male in question was disregarded.

One hundred eight males from the W-1 population were tested simultaneously for the presence of the lethal gene. A total of 11,042 offspring was counted. Six of the males were disregarded due to the afore-mentioned reasons. Of the remaining 102 tested males, 24 were heterozygous for the lethal gene. This yields an estimate of $Q = 0.2353 \pm 0.0420$ for the W-1 population, where $Q =$ the frequency of lethal heterozygotes.

The frequency of *l(2)55i* has, therefore, changed very little in three years ($Q = 0.34$ as compared to $Q = 0.2353 \pm 0.0420$) and has been maintained in this population under random mating conditions for more than four years.

### *Frequency of* l(2)55i *heterozygotes in the four experimental populations*

The frequency of *l(2)55i* heterozygotes in each of the four experimental populations was determined. In the generation before testing was to begin, 50 pairs of

flies were selected at random to begin the next generation. For the following five generations, 50 pairs were transferred every 15 days and at the time of transfer 15 males from each bottle were tested for the presence of the lethal. The testing method was the same as that described for testing the W-1 males. All flies were removed from each population bottle on the eighth day after introduction. Each bottle was analyzed as a possible subpopulation of the original population.

*Population 1:* Population 1 consists of seven bottles. Fifteen males from each bottle were tested in generations 57, 58, 59, 60 and 61. A total of 525 males was tested and 65 were disregarded due to insufficient offspring and/or the inability to distinguish between a 1:1 and 2:1 ratio.

The analysis of variance technique was applied to the estimates for $Q$ for each bottle in each generation. No significant differences in lethal heterozygote frequency ($Q$) were found between bottles or between generations. A single estimate of the frequency of lethal heterozygotes in Population 1 was obtained. Of the total 460 males, 88 were scored as being lethal heterozygotes. This gives an estimate of $Q = 0.1913 \pm 0.0194$ in Population 1 as shown in Table 3.

*Population 2:* Population 2 consists of seven bottles. Fifteen males from each bottle were tested in generations 46, 47, 48, 49 and 50. A total of 525 males was tested and 117 were disregarded due to insufficient offspring and/or the inability to distinguish between a 1:1 and 2:1 ratio.

This population was analyzed in the same way as Population 1. There was no significant difference in lethal heterozygote frequency between generations, but there was a highly significant difference between bottles. Because of this, an estimate of $Q$ for Population 2 as a whole was not calculated. Estimates of $Q$ for each of the seven subpopulations were obtained from the means of the five tested generations. These estimates are given in Table 3.

*Population 3:* Population 3 consists of eight bottles. Fifteen males from each bottle were tested in generations 33, 34, 35, 36 and 37 and, of the 600 males tested, 70 were disregarded due to insufficient offspring and/or inability to distinguish between a 1:1 and 2:1 ratio.

In this population there was no significant difference in $Q$ between bottles, but there was a significant difference between generations. This difference between generations was quite irregular and was in no way associated with a trend. Because of this, the difference was attributed to sampling variation and an estimate of $Q$ was made for Population 3 as a whole. Of the 530 males tested, 117 were heterozygous for the lethal. This yields an estimate of $Q = 0.2208 \pm 0.0195$ (Table 3).

*Population 4:* Population 4 consists of ten bottles. Fifteen males from each bottle were tested in generations 27, 28, 29, 30, and 31 and, of the 750 males tested, 146 were disregarded due to the afore-mentioned discrimination difficulties.

As in Population 2, there was no difference in lethal heterozygote frequency between generations, but there was a highly significant difference between bottles. An estimate of $Q$ for the entire population was not made. Estimates of the

TABLE 3

*Summary of information concerning Populations 1, 2, 3, 4 and W-1*

| | Genetic background | Lethal heterozygote freq. in starting generation | Est. equilibrium freq. gen. 7–16 | Equilibrium freq. of subpopulations as est. in this study | Pooled est. of equilibrium freq. with respect to subpop. and generations |
|---|---|---|---|---|---|
| Pop. 1 | W-1 | 100% | .420 ± .190 | .100 ± .028<br>.138 ± .055<br>.167 ± .053<br>.203 ± .077<br>.209 ± .014<br>.219 ± .042<br>.312 ± .035<br>(Gen. 57–61†) | .191 ± .019 |
| Pop. 2 | W-1 50%<br>W-109 50% | 100% | .424 ± .017 | .179 ± .040<br>.189 ± .096<br>.241 ± .038<br>.262 ± .036<br>.317 ± .055<br>.333 ± .094<br>.508 ± .058<br>(Gen. 46–50†) | ..... |
| Pop. 3 | Pop. 1 | 5%* | ..... | .156 ± .035<br>.167 ± .024<br>.177 ± .040<br>.227 ± .052<br>.228 ± .064<br>.254 ± .067<br>.262 ± .061<br>.286 ± .083<br>(Gen. 33–37†) | .221 ± .019 |
| Pop. 4 | Pop. 2 | 5%* | ..... | .129 ± .049<br>.143 ± .026<br>.164 ± .078<br>.246 ± .088<br>.300 ± .060<br>.339 ± .044<br>.441 ± .046<br>.441 ± .065<br>.569 ± .067<br>.571 ± .106<br>(Gen. 27–31†) | ..... |
| W-1 | ..... | .... | ..... | ..... | .235 ± .042 |

* Expected frequency.
† Each estimate is the mean of tests of these five generations.

frequency of the lethal heterozygote based on the means of the five tested generations for each of the ten subpopulations of Population 4 are given in Table 3.

Information about the four experimental populations and W-1 is summarized in Table 3. The four experimental populations differed as to genetic background

and lethal frequency in the starting generation. Populations 1 and 3 are similar in genetic background and Populations 2 and 4 are similar in genetic background. Populations 1 and 2 began with 100 percent lethal heterozygote ferquency whereas Populations 3 and 4 have an expected frequency of five percent at their origination. The results indicate that an important factor influencing lethal heterozygote frequency in these populations is the genetic background.

Populations 1 and 3 were derived from a single W-1 female and contain only the heterogeneity present in that female plus any spontaneous mutations which might have occurred since Population 1 was established. There were no heterogeneities between bottles in either of the populations with the W-1 genetic background.

Populations 2 and 4, on the other hand, are 50 percent W-1 and 50 percent W-109 in genetic background and would, therefore, be expected to have been more heterogeneous in the starting generations. Evidence of genetic difference is indicated by the fact that in both of these populations there were heterogeneities among the various subpopulations. This is to be expected since the greater amount of heterozygosity present in these populations allows for a greater chance of genetic segregation of genes other than the lethal. Therefore, the genetic background might be somewhat different from bottle to bottle in these populations. But, the lethal has not been lost, nor has it even reached a very low frequency in any subpopulation.

A t test was applied to the observed difference between $Q$ of Population 1 (0.191) and $Q$ of Population 3 (0.221). This difference was found to be not significant. A pooled estimate of the frequency of *l(2)55i* in these two populations with W-1 background yielded an estimate of $Q = 0.2071 \pm 0.0138$. The pooled estimates of $Q \pm$ one standard deviation in Populations 1 and 3 and in the W-1 stock overlap this value.

### *Factors involved in the expression of heterosis*

Studies concerning the various factors involved in the expression of overdominance of the gene *l(2)55i* have been carried out using Population 1(3–4), a derivative of Population 1. The results, therefore, apply only to populations with the W-1 background.

*Zygotic viability:* Evidence that there is no difference in zygotic viability (hatchability + larval competitive ability + length of larval period) between the wild type flies and flies heterozygous for *l(2)55i* was presented by MUKAI and BURDICK (1959). The relative zygotic viability of lethal heterozygotes was estimated by them at $0.974 \pm 0.032$ when Population 1(3–4) was used in the experiment. This result, then, applied to Populations 1 and 3. Additional evidence suggesting equal viability is the fact that in Population 1, starting at $Q = 1.00$, the second generation value of $Q(Q = 0.673)$ approximates the expected value $(Q = 0.667)$ based on no difference between the two types of flies in either zygotic viability or in sperm competitive ability.

*Sperm competitive ability:* Evidence indicating no difference between *l* and + sperm in sperm competitive ability (*s*) was cited in connection with zygotic viability. MUKAI and BURDICK (1959) also showed no difference in sperm competitive ability with direct experimentation. *Cy/l* females were mated to +/*l* males and an almost perfect 2:1 ratio of Curly to wild type was obtained in the offspring.

The possibility that *l*-carrying sperm might have a competitive advantage over +-carrying sperm was retested in this study.
Two types of crosses were made as follows:

| A | | B | |
|---|---|---|---|
| +/+ × *Cy/l* | | +/+ × *Cy*/+ (from +/+ × *Cy/l*) | |
| ↓ | | ↓ | |
| *Cy*/+ and +/l | | *Cy*/+ and +/+ | |
| (a) | (b) | (a) | (c) |

Because there is no difference between +/+ and +/*l* flies in zygotic viability, a comparison of the relative proportion of flies in the (b) and (c) classes of offspring will provide information concerning sperm competitive ability. Ten bottles (3-pair matings) of each of the two crosses were made. The offspring were counted daily for six days beginning on the tenth day.

A $\chi^2$ test for heterogeneity was applied to the data. The test indicated that the ten bottles within each cross were homogeneous. The pooled totals for each of the crosses are given in Table 4. The difference between the (b) and (c) classes is not significant. We can conclude that there is no difference between the competitive ability of *l*- and +-carrying sperm.

*Female fecundity:* Higher female fecundity may be responsible for the heterosis exhibited by *l(2)55i*. The fecundity of +/*l vs.* +/+ females was tested. Population 1(3–4) females were mated to C-101 males to obtain +/*l* males and females. The possibility that the type of male (+/+ or +/*l*) might affect fecundity was considered. The following four types of matings were made:

1. +/+ × +/+
2. +/+ × +/*l*
3. +/*l* × +/+
4. +/*l* × +/*l*

TABLE 4

*Pooled data for test of sperm competitive ability*

| Mating | | | |
|---|---|---|---|
| +/+ × *Cy/l* Offspring | | +/+ × *Cy*/+ Offspring | |
| *Cy*/+ | *l*/+ | *Cy*/+ | +/+ |
| (a) | (b) | (a) | (c) |
| 1245 | 1250 | 1314 | 1300 |
| $\frac{(b)}{(a)+(b)} = 0.50100$ | | $\frac{(c)}{(a)+(c)} = 0.49732$ | |
| $s = 1.0074$ | | $s = 1.0000$ | |

One female and two males were placed in each of 20 egg-laying bottles for each of the four crosses. Therefore, one replication of the experiment consisted of 80 egg-laying bottles and there were five replications. Two hundred +/+ and 200 +/*l* females were tested.

In the four experimental populations, the average effective egg-laying time was from age 60 hours to age 180 hours. In the test of fecundity the average age of females transferred to egg-laying caps was six hours. Any eggs deposited between this time and 48 hours later were not considered. Individual egg counts were made every 24 hours beginning on the third day and ending on the eighth day (effective egg-laying time from 54 to 198 hours). Fifty-two of the original 400 females were discarded for the following reasons: (1) Some of the females died. (2) Some lived but laid very few eggs. The fact that some laid very few eggs could have been due to either overetherization or a genetical inability to produce and/or deposit eggs. (3) Some of the females lived and deposited a high number of eggs in the early days but failed to lay in the later days of the experiment. This could have been due to an etherization effect, physical injury caused during cap transfer, or could have been a function of the genotype of the female.

The distribution by genotype of the discarded females is given in Table 5. Evidence in Table 5 indicates that approximately the same total number of each type of female was discarded but that the reasons for discarding were not similar for the two types of females. Eleven +/+ females were discarded because they laid very few eggs during the six days of count, but only two +/*l* females were discarded for this reason.

The egg-laying data were compiled on the basis of total eggs per female. Since the number of surviving females varied from cross to cross, the data were analyzed on the basis of the mean number of eggs per female for each of the four crosses within each replication. In order to test for an interaction between replication and type of mating, an error variance was calculated from the original per-female totals and corrected for use with the mean analysis. The analysis of the data is given in Table 6.

The mean square for type of female was highly significant. The mean square for interaction between type of female and replication was also highly significant. Because of the significance of this interaction, a single estimate of relative female

TABLE 5

*Distribution by genotype of females discarded in test of fecundity*

| Reason* | Type of female +/+ | +/*l* |
|---|---|---|
| 1 | 13 | 19 |
| 2 | 11 | 2 |
| 3 | 1 | 6 |
| Total | 25 | 27 |

* See text.

TABLE 6

*Analysis of variance in fecundity*

| Source | d.f. | Mean square | F |
|---|---|---|---|
| Replications | 4 | 3,628.98 | |
| Type mating | (3) | (9,587.18) | |
| A. Female | 1 | 28,656.23 | 18.268** |
| B. Male | 1 | 4.79 | |
| C. Female × male | 1 | 100.52 | |
| Replication × type mating | (12) | (1,568.63) | |
| A. Rep. × female | 4 | 3,729.75 | 12.900** |
| B. Rep. × Male | 4 | 133.14 | |
| C. Rep. × female × male | 4 | 843.00 | 2.928* |
| Total | 19 | | |
| Error (females/types/rep)† (corrected by use of harmonic mean of sample size) | 328 | 278.8150 | |

** Significant at the 0.01 level.
* Significant at the 0.05 level.
† Corrected by use of harmonic mean of sample size.

fecundity was not made. No apparent difference existed between the performance of the female when mated to the wild type and the performance of the female when mated to the lethal heterozygote. Therefore, the type of male was disregarded and mean values of number of eggs were calculated for the two types of females. These mean values are given in Table 7.

The data indicate that the highest estimated value of relative fecundity ($f$) of $+/l$ females ($+/+ = 1$) is 1.4030 and that the lowest estimate is 1.0192. Replications 1 and 3 yielded similar values of about 1.00 and replications 2, 4 and 5 gave values approximating 1.35. An explanation for this difference among replications cannot be made at this time. A total of 175 $+/+$ females produced 60,916 eggs. The mean number of eggs per $+/+$ female was 348.0914. The mean number of eggs per female for 173 $+/l$ females laying 72,881 eggs was 421.2774. The over-all $f$-value for $+/l$ females was 1.2102.

Since zygotic viability and sperm competitive ability were eliminated as possible factors influencing the heterotic effect of *l(2)55i*, the relationship of $f$, $m$,

TABLE 7

*Mean values of number of eggs in five replications of four types of matings*

| Replication | Female $+/+$ (a) | Female $+/l$ (b) | Relative fecundity (b)/(a) = $f$ |
|---|---|---|---|
| 1 | 338.46 | 347.69 | 1.0273 |
| 2 | 350.86 | 460.78 | 1.3133 |
| 3 | 366.97 | 374.03 | 1.0192 |
| 4 | 331.62 | 465.25 | 1.4030 |
| 5 | 356.35 | 479.33 | 1.3451 |

and $Q$ were considered. According to formulae derived by MUKAI and BURDICK (1959), the following relationships exist where: $m$ = relative mating ability of $+/l$ males, that of $+/+$ males being 1; $f$ = relative fecundity of $+/l$ females, that of $+/+$ females being 1, and $Q$ = equilibrium frequency of lethal heterozygotes.

$$m = [4(1-Q)^2 + 2(1-Q)(2Q-1)f] \,/\, [2(1-Q)(1-2Q) + Q(2-3Q)f] \quad \text{.....(1)}$$

If $m = 1$ and only fecundity is involved,

$$f = [2(1-Q)] \,/\, [Q^2 - 4Q + 2] \quad \text{.....(2)}$$

If $f = 1$ and only mating ability is involved,

$$m = [2(1-Q)] \,/\, [Q^2 - 4Q + 2] \quad \text{.....(3)}$$

The estimated $Q$ for populations with W-1 genetic background is 0.2071 ± 0.0138. If 0.2071 is a true equilibrium and only one factor is involved, $f$ (or $m$) must be equal to 1.3057 according to Formulae (2) and (3). Estimated values of $f$ and theoretical values of $m$ are given in Table 8. Column 1 in the table shows the pooled minimum and single maximum values of $f$ derived from this experiment. Column 2 indicates the theoretical value of $Q$ which the corresponding $f$ value will support, assuming $m = 1$ (Formula 2). Column 3 shows the actual value of $Q$ as estimated from populations with W-1 background in the previous experiments. Column 4 shows the theoretical value of $m$ when Columns 1 and 3 are assumed true (Formula 1).

*Male mating ability:* The relative mating ability of $+/l$ males ($+/+$ males = 1) was tested in this study. From a cross of Population 1 (3–4) and C-101, $+/l$ and $+/Cy$ males were obtained. The following two types of crosses were made.

$$\begin{array}{cc} \text{A} & \text{B} \\ +/+ \times (+/l \text{ and } +/Cy) & +/+ \times (+/Cy \text{ and } +/+) \\ \downarrow & \downarrow \\ (+/+ \text{ and } +/l) \text{ and } +/Cy & +/+ \text{ and } +/Cy \end{array}$$

The $+/+$ flies in both crosses were from Population 1 (3–4). Since it was indicated that there was no difference between $+/+$ and $+/l$ in zygotic viability nor in sperm competitive ability, mating ability ($m$) is the only effective factor difference in the above experiment.

Fifty females and 25 of each type of male were put into each of ten bottles. Five bottles were of mating A and five were of mating B. If the proportion of phenotypically wild type flies (a) from mating A is compared to the proportion of wild type flies (b) from mating B the result (a)/(b) is a function of relative

TABLE 8

*Estimated* f *and corresponding values of* m *and* Q

| (1) $f$ | (2) Calculated $Q$ when $m = 1$ | (3) Estimated $Q$ | (4) Theoretical value of $m$ |
|---|---|---|---|
| (min.) 1.2102 | 0.1587 | 0.2071 | 1.0910 |
| (max.) 1.4030 | 0.2470 | 0.2071 | 0.9112 |

mating ability of $+/l$ males when mating ability of $+/+$ males = 1. One bottle from mating B was discarded.

There were no heterogeneities among bottles in mating A, but there were heterogeneities among bottles in mating B. A possible explanation for the above is the fact that in mating A the two types of males were the result of a single etherization whereas in mating B the two types of competing males were the result of two separate etherizations. Five single estimates and a pooled estimate of (a) and four single estimates of (b) are given in Table 9.

The most interesting comparison in this data is a comparison of the pooled estimate of (a) with the lowest estimate of (b), $m = 1.0289$. A significant difference in these two values (0.7628 and 0.7414) would suggest that the mating ability of $+/l$ males was better than that of $+/+$ males. A test indicated no significant difference between the two values. Therefore, the mating ability of $+/l$ flies is probably not greater than that of $+/+$ flies. If anything, the data show poorer mating ability of $+/l$ males.

The earlier equilibrium of 0.42 reported by MUKAI and BURDICK (1959) was evidently supported by superior female fecundity ($f$) and male mating ability ($m$) of lethal heterozygotes. Now, $m$ appears to have dropped to 1.0 and the equilibrium has been reduced to 0.20. If we assume that $m$ and $f$ have experienced proportionate reductions, we can estimate that, at the time when equilibrium was 0.42, $f$ was 1.70 and $m$ was 1.44.

### *Changes in equilibrium frequency of lethal heterozygotes*

As can be noted in Table 3, MUKAI and BURDICK (1959) found that their lethal heterozygous populations (Populations 1, 2, 3, and 4) reached an equilibrium where the relative frequencies of lethal heterozygotes ($Q$) were all about 0.42. The data presented in this report clearly show a lower equilibrium of lethal heterozygotes (about 0.20). This decrease, which took place over a period of 16 to 40 generations, may be due to the effect of mutations in the genetic background modifying the relative viability of lethal heterozygotes. Since the superviability of the lethal heterozygote is attributable to only one factor, female fecundity, the

TABLE 9

*Proportion of wild type flies resulting from test of mating ability*

| | Proportion of wild type flies from cross A (a) | Proportion of wild type flies from cross B (b) |
|---|---|---|
| | 0.7208 | 0.7414 |
| | 0.7558 | 0.7855 |
| | 0.7600 | 0.8230 |
| | 0.7754 | 0.8244 |
| | 0.7991 | |
| Pooled estimate | 0.7628 | |

mathematical model showing the effect of heterozygote-viability-modifying mutations may be developed.

MUKAI and BURDICK (1959) showed the following relationship in an equilibrium lethal heterozygote population:

$$f(\text{or } m) = \frac{2(1-Q)}{Q^2-4Q+2} \quad \text{.....(2)}$$

where $f$ stands for the relative female fecundity and $m$ for the relative male mating ability of lethal heterozygotes, assuming that the $m$'s and $f$'s of wild types are 1.00, and $Q$ stands for the equilibrium frequency of the lethal heterozygotes.

The following expression can be obtained from Formula (1):

$$Q = 2 - (1/f)\ [1 \pm \sqrt{2(f-\tfrac{1}{2})^2 + \tfrac{1}{2}}] \quad \text{.....(4)}$$

The negative root of formula (2) can be disregarded because $Q$ should be between 0 and 1. Thus,

$$Q = 2 - (1/f)\ [1 + \sqrt{2(f-\tfrac{1}{2})^2 + \tfrac{1}{2}}] \quad \text{.....(5)}$$

If we substitute $R$ for $(1/f)\ [1 + \sqrt{2(f-\tfrac{1}{2})^2 + \tfrac{1}{2}}]$, the zygotic array in the equilibrium population can be expressed as $(AA, Aa) = (R-1, 2-R)$, where "$a$" denotes the recessive lethal gene in question.

If a lethal-heterozygote-viability-modifying mutation takes place in the genetic background, it causes a change in the relative female fecundity from $f$ to $(f + \Delta f)$. The frequency of individuals that have one or another of such new modifying mutations is $c$. This produces the following zygotic array:

$$(AA, {}_{\alpha}Aa, {}_{\beta}Aa) = [R-1,\ (2-R)\ (1-c),\ (2-R)c]$$

where $\alpha$ is the original genetic background and $\beta$ the new genetic background carrying the mutation.

After one random mating generation, the frequency of the lethal heterozygotes would become:

$$Q' = \frac{R^2(2-R)\ (R-1) + (2-R)\ (R^2-2)\ \Delta fc}{R^2(R-1) + \Delta fc(2-R)\ (\tfrac{1}{2}R+1)\ (R^2-2)} \quad \text{.....(6)}$$

The increment of lethal heterozygote frequency $(\Delta Q)$ is expressed by the following formula:

$$\Delta Q = Q' - Q$$

$$= \frac{\Delta fc(2-R)\ (R^2-2)^2}{2[R^2\ (R-1) + \Delta fc(2-R)\ (R^2-2)\ (\tfrac{1}{2}R+1)]} \quad \text{.....(7)}$$

And, when $f > 1$,

$$(2-R) = \frac{2(f-1)}{[2f-1+\sqrt{2(f-\tfrac{1}{2})^2+\tfrac{1}{2}}]} > 0$$

$$(R^2-2) = \frac{2}{\sqrt{2(f-\tfrac{1}{2})^2+\tfrac{1}{2}}+(f-1)} > 0$$

Therefore, every term in Formula (7) is positive except $\Delta f$.

If we assume that an equal number of positive and negative mutations takes place, each having the same magnitude of effect, then the absolute values of the

numerator in Formula (7) are the same for a positive and a negative mutation. However, the denominator becomes smaller for a negative mutation and larger for a postive mutation making the positive effect on $Q$ always less than the negative effect. Thus, a lethal gene has a destiny of elimination from the population by random spontaneous mutational changes of modifying genes even though the lethal heterozygote shows superviability associated with female fecundity ($f$). The same conclusion can be reached in the case of superviability associated with mating ability of males ($m$) because Formula (2) applies to both $m$ and $f$.

Other factors which may have influenced the change in equilibrium frequency of lethal heterozygotes are: (1) Change in mating system. There was no intergeneration mating prior to the 16th generation in any of the four populations; there was intergeneration mating after that time. (2) Change in population size. The first 15 generations of each population began with 50 pairs of flies. The following generations began with more than 50 pairs. This would increase population size within the bottles and lead to greater competition between genotypes.

## SUMMARY AND CONCLUSIONS

Various investigations concerning the lethal gene *l(2)55i* have been carried out in this study. Since this gene has previously been shown to exhibit heterosis (overdominance), an attempt has been made to determine how this heterotic effect is expressed.

1. Homozygous *l(2)55i* produces its lethal effect at some time during the larval period.

2. The gene *l(2)55i* was first found in, and extracted from, the wild type stock W–1 where the frequency of the heterozygote was about 0.34. The present test of lethal heterozygote frequency in W–1 yielded an estimate of $0.2353 \pm 0.0420$. This lethal has been maintained in this stock by random mating for more than four years.

3. Four experimental populations originally established in 1956 were tested for the present frequency of the lethal heterozygote. These populations differed from one another with regard to genetic background and initial lethal gene frequency. These four populations were found to have segregated from one another on the basis of genetic background. The two populations having W–1 genetic background were found to be fairly uniform in lethal gene frequency from bottle to bottle within each of the two populations, whereas the two populations having a mixed genetic background were found to have a variable lethal gene frequency from bottle to bottle within each of the two populations. Nevertheless, no bottle in any of the populations had lost nor nearly lost the lethal. The various estimates of the frequency of the lethal heterozygotes in the four populations were as follows:

Population 1, $0.1913 \pm 0.0194$; Population 2, seven estimates ranging from $0.1786 \pm 0.0398$ to $0.5078 \pm 0.0578$; Population 3, $0.2207 \pm 0.0195$; Population 4, ten estimates ranging from $0.1290 \pm 0.0493$ to $0.5714 \pm 0.1063$.

The estimates of lethal gene frequency in Populations 1 and 3 did not differ

significantly. A pooled estimate of lethal gene frequency in these two populations having W–1 genetic background is 0.2071 ± 0.0138. This pooled estimate is very near the lethal heterozygote frequency in W–1 (0.2353 ± 0.0420).

The equilibrium frequency of lethal heterozygotes was found to have changed from about 0.42 to about 0.20 over a period of 16 to 40 generations. This change can be attributed, at least in part, to random spontaneous mutational changes of modifying genes.

4. Experiments concerning the factors involved in the expression of heterosis were carried out using a homozygous wild type derivative of Population 1. Since this population has the W–1 genetic background, the results apply only to those populations with that background.

a. Zygotic viability was shown previously to be the same for lethal heterozygotes and the wild type. This fact was substantiated in this study.

b. Sperm competitive ability was shown to be the same for *l*-carrying and +-carrying sperm. This fact substantiates previous work.

c. The relative fecundity of $+/l$ females was measured giving two estimates of relative fecundity as follows:

1. 1.2102
2. 1.4030

The higher estimate of fecundity would be more than sufficient to support the equilibrium value of $Q$, 0.2071, while the lower estimate would require that the relative mating ability of $+/l$ males (when $+/+ = 1$) be 1.0910.

d. Experiments concerning the relative mating ability of $+/l$ males indicated that this value was not greater than unity.

The results of the above experiments indicate that the heterotic effect of the gene *l(2)55i* as expressed in those populations having the W–1 genetic background is due almost entirely to the greater fecundity of the lethal heterozygous females.

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# POSTIRRADIATIVE EFFECTS ON CHROMOSOMAL ABERRATIONS IN TRADESCANTIA MICROSPORES[1]

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SINCE 1939 when SAX employed the time-intensity factor to determine how long a chromosome break would remain open and thus be available for interaction with other breaks, many different methods of influencing X-ray-induced chromosomal aberrations have been used in studying the rejoining capacity of broken chromosomes. SAX concluded that high-intensity radiation is more effective in producing aberrations than low and that continued radiation is more effective than intermittent. FANO and MARINELLI (1943) concluded from some of their work that fractionation experiments consisting of two high-intensity irradiations separated by a variable time interval was better suited to investigations of the time-intensity factor than experiments in which the intensity of continuous irradiation was varied. More recently this approach has been used by COHN (1958), WOLFF and LUIPPOLD (1958) and others. In addition to studies involving the method of irradiation, following THODAY and READ'S (1947) discovery of the oxygen effect, the use of environmental variations has provided valuable information concerning the rejoining capacity of X-ray-induced chromosome breaks. The investigations of MAZIA (1954), STEFFENSEN (1955) and WOLFF and LUIPPOLD (1956) have indicated that ionic bonds may play a role in chromosome organization, while the work of WOLFF and LUIPPOLD (1955) and BEATTY and BEATTY (1957) indicates that energy supply is an important factor in the rebonding of the chromosome breaks.

Previous investigations (BEATTY and BEATTY 1957, 1959 and BEATTY, BEATTY, and COLLINS 1956) revealed that a total dose of 400r of X-radiation at intensities from 1r to 400r per minute, in combination with various environmental factors during or after irradiation, yielded in five percent oxygen an average low of 0.48 and a high of 0.88 chromosomal aberrations per cell, while in helium a low of 0.14 and a high of 0.66 was found. In those experimental procedures in which, with the same dose of 400r, less than the maximum yield of aberrations was obtained, by modifying the experimental setup so as to influence the rejoining of broken chromosome ends, a yield up to but not greater than the high could be obtained. The results indicated in all of these investigations that a total dose of 400r in helium produced a constant number of recoverable chromosome breaks and that in five percent oxygen the same was true although differing quantitatively from that in helium. This idea of constancy in number of recoverable

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breaks per given radiation dose, along with the role which available energy plays in radiation recovery, formed the basic premise for the investigations reported here.

## MATERIAL AND METHODS

Inflorescences of *Tradescantia paludosa* were removed just prior to use from plants growing in the greenhouse and after treatment returned to the greenhouse in beakers of water, where they remained until acetocarmine preparations of the microspores were made 96 hours later. Chromosomal aberrations of the ring and dicentric types were scored .The data are recorded as average aberrations per cell, and the standard errors calculated by the method discussed by CATCHESIDE, LEA, and THODAY (1946).

The experimental temperatures used were 0.3°C, 30°C and 40°C. In the two temperature extremes, the material was allowed 40 minutes for adaptation. Due to the construction of the lead cage around the X-ray unit, all experiments are normally carried out in the dark since all equipment and all connections for manipulating gas exchanges are exterior to the protective shield.

The basic rate of irradiation was 50r per minute except in those data reported in Table 1, where 400r per minute was used. A total dose of 400r was used except in those cases where half-dose data were needed. In all fractionation studies two doses of 200r each were used.

No pretreatment period was employed for the various gases, but a posttreatment period of 15 minutes was used in each experiment following the total dose of X-radiation. In the fractionation experiments the gas used and the length of time used between fractions is given for each experiment in the tables with the experimental results.

The total volume of the exposure chamber and the tubing used in making connections is small. Vacuum down to a few millimeters of mercury can be obtained in this equipment in 20 to 25 seconds. In any experiment involving postirradiation change from air to helium, each of the first two evacuations and gas replacement

TABLE 1

*Posttreatment effects of carbon monoxide after 400r of X-radiation given at 400r per minute at 30°C and posttreatment continued for 15 minutes*

| Experiment no. | Irradiation atmosphere | Minutes CO admitted after irradiation | No. of cells | Av. interchanges per cell |
|---|---|---|---|---|
| 1 | 5% $O_2$ in He | ..... | 800 | 0.55 ± 0.03 |
| 2 | 5% $O_2$ in CO | ..... | 1000 | 0.86 ± 0.03 |
| 3 | 5% $O_2$ in He | 32 sec | 1000 | 0.86 ± 0.03 |
| 4 | " | 5 min | 500 | 0.64 ± 0.04 |
| 5 | " | 10 min | 500 | 0.56 ± 0.03 |
| 6 | " | 15 min | 500 | 0.56 ± 0.03 |
| 7 | " | 20 min | 500 | 0.56 ± 0.03 |
| 8 | " | 30 min | 500 | 0.57 ± 0.03 |

required about 30 seconds. For the third evacuation 50 seconds were used plus a few seconds for gas replacement, so that the three exchanges required a total of two minutes plus or minus a few seconds. If the gas exchange was from helium to air, only one evacuation of 1.5 minutes was used for the air replacement. The CO gas employed was bubbled through two solutions of alkaline pyrogallate.

## RESULTS

Since the use of carbon monoxide to inhibit cytochrome oxidase thereby increasing radiation damage is well-known, this technique was employed in several experiments using Tradescantia microspores. The material was given a one minute exposure to X-radiation at an intensity of 400r per minute in five percent oxygen. The accompanying gas was either helium or CO. With helium (experiment 1, Table 1) an aberration yield per cell of 0.55 was found, while in CO, in the dark, a yield of 0.86 (experiment 2) was obtained. The CO effect was light reversible. In experiment 3 the X-ray exposure was in five percent oxygen in helium but immediately following irradiation the exposure chamber was evacuated and CO was admitted sufficiently to restore atmospheric pressure. This procedure required 32 seconds. The aberration count was found to be 0.86 per cell, the same as in experiment 2 where CO was present during irradiation. These data demonstrate, firstly, a postirradiation effect and, secondly, indicate that the CO effect was operative on the recovery or rejoining mechanism rather than on breakage. Another observation worthy of consideration is the rapidity with which the CO effect was initiated. The transportation of CO to the crucial sites in the cell, the inhibitory expression and the blockage of the flow of energy to the damaged areas appeared to be exceedingly rapid.

In Tradescantia an induced delay in the chromosome rejoining process, whether it be accomplished by chemical or physical means, increases the yield of two-hit aberrations. The inhibition of the cytochrome system by CO apparently increases the yield by bringing about a delay in rejoining due to an insufficient amount of available energy. Keeping this in mind, along with the conclusions drawn from experiments 2–3, it appeared feasible to determine how long after irradiation (with oxygen present in the cell so that energy might be available for rejoining) a CO effect might be produced. This would give information on the length of time the broken ends of the chromosomes are available after radiation was administered for two-hit aberration production. In experiments 4–8, irradiation was given in five percent oxygen in helium and the material remained in the gas mixture for various periods of time following irradiation, after which the gases were replaced with CO. If energy is available, it appears that much chromosome rejoining, whether it be restitution or the formation of new reunions, takes place during the first five minute period following irradiation (experiment 4). Experiments 5–8, when compared with 1, indicate that after ten minutes such rejoining has taken place so that there is no CO effect.

A second method employed to modify the flow of energy within the cell was to incorporate anoxic conditions in the experimental design, thereby inhibiting

oxidative metabolism, which in turn reduced the amount of energy available for chromosome rejoining. By using various sequences of gases, this procedure provided another means of studying postirradiation effects.

Each of the first three experiments, the results of which are recorded in Table 2, was given a total dose of 200r at 50r per minute in air. In experiment 9 (posttreatment in air for 15 minutes) the result was 0.23 aberrations per cell. Following posttreatment in helium for the same period of time, an aberration yield of 0.30 was recorded, while with 60 minutes posttreatment in helium 0.39 aberrations per cell were scored. Similarly, in the experiments using a total dose of 400r, more aberrations were recorded following posttreatment in helium, 0.94, than in air, 0.79.

The results of the companion experiments to those reported in Table 2 are given in Table 3. In these irradiation was done under conditions of anoxia and the posttreatment was either in air or in a continuation of the anoxic state. In experiment 14 a total dose of 200r with posttreatment in helium yielded 0.20 aberrations per cell, while posttreatment in air gave 0.11. In a similar manner a total dose of 400r gave 0.66 aberrations when posttreated in helium, but only 0.26 when posttreated in air. The admittance of air to the material immediately following irradiation allowed for the oxidative processes to operate, thus providing immediate energy in the cell which favored restitution over new reunions and reduced the aberration yield.

TABLE 2

*The effects of posttreatment in different gases with X-irradiation at 50r per minute in air at 30°C*

| Experiment no. | Dose in air | Posttreatment in minutes | | No. of cells | Av. interchanges per cell |
|---|---|---|---|---|---|
| | | Air | Helium | | |
| 9 | 200r | 15 | .. | 1100 | 0.23 ± 0.02 |
| 10 | 200r | .. | 15 | 300 | 0.30 ± 0.03 |
| 11 | 200r | .. | 60 | 400 | 0.39 ± 0.03 |
| 12 | 400r | 15 | .. | 1890 | 0.79 ± 0.02 |
| 13 | 400r | .. | 60 | 450 | 0.94 ± 0.04 |

TABLE 3

*The effects of posttreatment in different gases with X-irradiation at 50r per minute in helium at 40°C*

| Experiment no. | Dose in helium | Posttreatment in minutes | | No. of cells | Av. interchanges per cell |
|---|---|---|---|---|---|
| | | Air | Helium | | |
| 14 | 200r | .. | 15 | 1000 | 0.20 ± 0.01 |
| 15 | 200r | 15 | .. | 500 | 0.11 ± 0.02 |
| 16 | 400r | .. | 15 | 500 | 0.66 ± 0.04 |
| 17 | 400r | 15 | .. | 500 | 0.26 ± 0.02 |

Since the experimental results recorded in the first three tables indicate a posttreatment differential, depending on whether oxygen was present or absent in the experiment, a procedure was devised to test the capacity of the chromosomes to rejoin and the length of time during which rejoining of breaks produced in air and in helium takes place. To test these factors, fractionated doses of X-radiation were used, with the gas and the time interval between doses varied in order to determine under what conditions the two doses were synergistic and when they became independent. The results are given in Tables 4 and 5 in experiments 18–50.

The intensity in all experiments was 50r per minute so that the 200r exposure was given in four minutes in each case. No pretreatment was used so that dose I was always given as soon as the experimental conditions were completed. In experiments 18–24 air was used as the gas during irradiation, during the intervals between doses and for posttreatment. A 200r dose yielded 0.23 aberrations per cell, while a 400r dose gave 0.79. The time intervals of 5, 10 and 15 minutes between doses showed progressively fewer aberrations than recorded in experiment 19 where no time interval existed between fractions. The independent or additive aspect of the two doses was evident first at the 30-minute interval and also present at the one-hour interval, since 0.47 and 0.45 aberration yields are approximately twice the value of 0.23 obtained in experiment 18 where the single dose was used.

In experiments 25–32 the X-radiation for both doses was given in helium, but posttreatment following dose I was in air for comparative purposes with experiments 20–24 while the second dose was posttreated in helium. This helium post-

TABLE 4

*Fractionation experiments using air or helium during irradiation with air between doses and X-irradiation at 50r per minute at 30°C*

| Experiment no. | Gas dose I | Posttreatment | | Gas dose II | Posttreatment | | No. of cells | Av. interchanges per cell |
|---|---|---|---|---|---|---|---|---|
| | | Min. | Gas | | Gas | Min. | | |
| 18 | air 200r | 15 | air | ..... | .. | .. | 1100 | 0.23 ± 0.02 |
| 19 | air 400r | 15 | air | ..... | .. | .. | 1890 | 0.79 ± 0.02 |
| 20 | air 200r | 5 | air | air 200r | air | 15 | 500 | 0.74 ± 0.04 |
| 21 | air 200r | 10 | air | air 200r | air | 15 | 500 | 0.71 ± 0.04 |
| 22 | air 200r | 15 | air | air 200r | air | 15 | 800 | 0.55 ± 0.03 |
| 23 | air 200r | 30 | air | air 200r | air | 15 | 800 | 0.47 ± 0.02 |
| 24 | air 200r | 60 | air | air 200r | air | 15 | 600 | 0.45 ± 0.03 |
| 25 | He 200r | 15 | He | ..... | .. | .. | 1250 | 0.07 ± 0.01 |
| 26 | He 200r | 15 | air | ..... | .. | .. | 500 | 0.08 ± 0.01 |
| 27 | He 400r | 15 | He | ..... | .. | .. | 1500 | 0.23 ± 0.01 |
| 28 | He 200r | 5 | air | He 200r | He | 15 | 500 | 0.18 ± 0.02 |
| 29 | He 200r | 10 | air | He 200r | He | 15 | 500 | 0.20 ± 0.02 |
| 30 | He 200r | 15 | air | He 200r | He | 15 | 500 | 0.19 ± 0.01 |
| 31 | He 200r | 30 | air | He 200r | He | 15 | 200 | 0.15 ± 0.03 |
| 32 | He 200r | 60 | air | He 200r | He | 15 | 550 | 0.15 ± 0.02 |

TABLE 5

*Fractionation experiments using air or helium during irradiation with helium as the gas between doses and X-irradiation at 50r per minute at 30°C*

| Experiment no. | Gas dose I | Posttreatment | | Gas dose II | Posttreatment | | No. of cells | Av. interchanges per cell |
|---|---|---|---|---|---|---|---|---|
| | | Min. | Gas | | Gas | Min. | | |
| 33 | air 200r | 15 | air | ..... | .. | .. | 1100 | 0.23 ± 0.02 |
| 34 | air 200r | 15 | He | ..... | .. | .. | 300 | 0.30 ± 0.03 |
| 35 | air 200r | 60 | He | ..... | .. | .. | 400 | 0.39 ± 0.03 |
| 36 | air 400r | 15 | air | ..... | .. | .. | 1890 | 0.79 ± 0.02 |
| 37 | air 200r | 5 | He | air 200r | air | 15 | 300 | 0.79 ± 0.05 |
| 38 | air 200r | 10 | He | air 200r | air | 15 | 500 | 0.77 ± 0.04 |
| 39 | air 200r | 15 | He | air 200r | air | 15 | 500 | 0.75 ± 0.04 |
| 40 | air 200r | 30 | He | air 200r | air | 15 | 600 | 0.62 ± 0.03 |
| 41 | air 200r | 60 | He | air 200r | air | 15 | 500 | 0.59 ± 0.03 |
| 42 | He 200r | 15 | He | ..... | .. | .. | 1250 | 0.07 ± 0.01 |
| 43 | He 400r | 15 | He | ..... | .. | .. | 1500 | 0.23 ± 0.01 |
| 44 | He 200r | 5 | He | He 200r | He | 15 | 500 | 0.22 ± 0.02 |
| 45 | He 200r | 10 | He | He 200r | He | 15 | 500 | 0.22 ± 0.02 |
| 46 | He 200r | 15 | He | He 200r | He | 15 | 500 | 0.22 ± 0.02 |
| 47 | He 200r | 30 | He | He 200r | He | 15 | 500 | 0.22 ± 0.02 |
| 48 | He 200r | 60 | He | He 200r | He | 15 | 500 | 0.20 ± 0.02 |
| 49 | He 200r | 90 | He | He 200r | He | 15 | 300 | 0.24 ± 0.03 |
| 50 | He 200r | 120 | He | He 200r | He | 15 | 300 | 0.30 ± 0.03 |

treatment allows a condition of anoxia to develop, thus reducing energy for rejoining and thereby holding open the breaks so that there would be an optimum opportunity for the interaction of breaks produced by both doses. The 200r single dose experiments, when posttreated in air or helium for 15 minutes, gave almost the same aberration yield, these being 0.07 in helium and 0.08 in air. In the fractionated experiments, the time intervals of 5, 10 and 15 minutes between doses resulted in progressively decreasing aberration yields when compared with results obtained in experiment 27. The independent or additive effect of the two doses appeared at the 30-minute interval and continued through the one-hour period, as indicated by experiments 31 and 32, where the yield of 0.15 aberrations equals the sum of experiments 25 and 26.

It appears from these two sets of experiments, 18–24 and 25–32, that chromosome breaks made in air or helium remain active about the same length of time. Regardless of whether the breaks produced by X-radiation were made in an atmosphere of air or helium, when the recovery period for the first dose was in air, the independence of the two doses or the additive nature was observed first in material which had an interim of 30 minutes between doses.

In Table 5 the results of parallel experiments to those reported in Table 4 are given, in which the irradiation was done either in air or helium, but the posttreatment period following dose I took place in helium. Since the anoxic condition produced by helium inhibits oxidative metabolism, thereby drastically reducing the energy available for chromosome rejoining, breaks produced during

the first irradiation should remain open for a longer time in an intervening helium atmosphere than when air was used between doses (Table 4). In experiments 37–41 the first dose of X-radiation was given in air followed by a period in helium. The second fraction was given in air followed by posttreatment in air. Because the posttreatment influences the aberration yield, as demonstrated by data in Tables 2 and 3, one must consider whether the posttreatment was in air or helium in determining the fractionation period when the effect of the two doses becomes additive. Experiment 33 gives the results in this series of experiments when X-radiation was followed by air and 34 and 35 when posttreatment was in helium. Experiment 36 gives the maximum number of aberrations expected in this series of experiments, 0.79, since the total dose was administered at one time. Five minutes intervening between doses of X-radiation (experiment 37) gave the same results as was found in experiment 36. Experiments 38–40 show progressively fewer aberrations as the period between doses increased from ten through 15 and 30 minutes. At the 60-minute period the results seem to be additive when compared to the summation results of experiments 33 and 35.

In experiments 44–50 both exposures and the periods following irradiation were in helium. In experiment 42 the results from a single 200r dose are recorded, 0.07, while experiment 43 gives the yield of 0.23 from a 400r dose. As the time between doses increased from five minutes to 10, 15, 30, 60 and 90 minutes, the aberration yield remained relatively constant. At the two-hour period an increase to 0.30 was found and was anticipated, since previous results (BEATTY and BEATTY 1959) indicated that at this temperature this length of time under anoxic conditions thusly affected aberration yield. The *status quo* maintenance of breaks as expressed in experiments 44–49 has provided a useful approach for studying the postirradiative effects of various chemical and physical agents. To a lesser extent the information obtained from experiments 37–41 is being used in a similar manner, although a change in procedure to higher intensities seemed warranted, since at 50r per minute four minutes are required to administer the dose, and the breaks in air have this period in which some rejoining can take place. This is possibly the reason that the results of the two doses in experiment 41 are additive at the one-hour intervening period.

Results from previous investigations (BEATTY and BEATTY 1959) have shown that temperature from zero degrees to 45°C, when used in combination with X-radiation administered at 50r per minute for eight minutes, modified the aberration yield. This was true for material irradiated in an oxygen atmosphere as well as in helium. In helium irradiation at 30°C yielded 0.23 aberrations per cell, while an exposure at 40°C resulted in an increase to 0.66 aberrations. In a five percent oxygen atmosphere the highest yield was at 0.3°C. The experimental results reported in Tables 6 and 7 incorporate these temperature extremes and the fractionation procedure.

In Table 6 the experiments were carried out in helium at 40°C. In experiment 51 material given a single 200r exposure to X-radiation, when posttreated in helium, yielded 0.20 aberrations per cell, while following posttreatment in air

TABLE 6

*Fractionation experiments with irradiation in helium atmosphere and posttreatments either in air or helium with X-irradiation at 50r per minute at 40°C*

| Experiment no. | Gas dose I | Posttreatment | | Gas dose II | Posttreatment | | No. of cells | Av. interchanges per cell |
|---|---|---|---|---|---|---|---|---|
| | | Min. | Gas | | Gas | Min. | | |
| 51 | He 200r | 15 | He | ..... | .. | .. | 1000 | 0.20 ± 0.01 |
| 52 | He 200r | 15 | air | ..... | .. | .. | 500 | 0.11 ± 0.02 |
| 53 | He 400r | 15 | He | ..... | .. | .. | 500 | 0.66 ± 0.04 |
| 54 | He 200r | 30 | He | He 200r | He | 15 | 500 | 0.68 ± 0.04 |
| 55 | He 200r | 5 | air | He 200r | He | 15 | 400 | 0.47 ± 0.03 |
| 56 | He 200r | 10 | air | He 200r | He | 15 | 700 | 0.45 ± 0.03 |
| 57 | He 200r | 15 | air | He 200r | He | 15 | 500 | 0.32 ± 0.03 |
| 58 | He 200r | 30 | air | He 200r | He | 15 | 500 | 0.33 ± 0.03 |

TABLE 7

*Fractionation experiments with irradiation at 50r per minute given in air at 0.3°C with posttreatments either in helium or air*

| Experiment no. | Gas dose I | Posttreatment | | Gas dose II | Posttreatment | | No. of cells | Av. interchanges per cell |
|---|---|---|---|---|---|---|---|---|
| | | Min. | Gas | | Gas | Min. | | |
| 59 | air 200r | 15 | air | ..... | .. | .. | 1000 | 0.33 ± 0.02 |
| 60 | air 400r | 15 | air | ..... | .. | .. | 500 | 1.07 ± 0.05 |
| 61 | air 200r | 5 | air | air 200r | air | 15 | 500 | 0.93 ± 0.04 |
| 62 | air 200r | 10 | air | air 200r | air | 15 | 500 | 0.87 ± 0.04 |
| 63 | air 200r | 15 | air | air 200r | air | 15 | 500 | 0.83 ± 0.04 |
| 64 | air 200r | 30 | air | air 200r | air | 15 | 500 | 0.82 ± 0.04 |
| 65 | air 200r | 5 | He | air 200r | air | 15 | 500 | 0.87 ± 0.04 |
| 66 | air 200r | 10 | He | air 200r | air | 15 | 500 | 0.91 ± 0.04 |
| 67 | air 200r | 15 | He | air 200r | air | 15 | 500 | 0.85 ± 0.04 |
| 68 | air 200r | 30 | He | air 200r | air | 15 | 500 | 0.86 ± 0.04 |

the yield was only 0.11. When both doses were given without any time intervening, a yield of 0.66 was obtained. When a helium atmosphere was maintained throughout the experiment, as in 54, a 30-minute interval between exposures did not alter the aberration yield from that obtained when the total dose was given at one time, experiment 53. When air was used as the gas intervening between doses of radiation, experiments 55–58, a decrease in aberration yield was observed at the 5, 10 and 15-minute periods and at the 30-minute period the results from the two exposures became additive.

The experimental results reported in Table 7 were obtained from material exposed to X-radiation in air at 0.3°C. A 200r exposure gave 0.33 aberrations per cell, while a 400r exposure yielded 1.07 aberrations. Periods of 5, 10, 15 and 30 minutes between doses were used. In experiments 61–64 air was used as the atmosphere in the intervening periods, while in experiments 65–68 helium was used. The aberration yield recorded for experiments 61–68 did not vary to any

great extent, although in those experiments where helium was used the aberration yield tended to be slightly higher. The similarity in results from experiments 61–68 might be explained on the basis of enzyme inactivation at such a low temperature.

## DISCUSSION

Much emphasis in investigations during the past few years dealing with the biological effects of radiation has been placed on discovering protective agents against radiation damage. The individual investigators, interested primarily in the protective nature, were not concerned necessarily with the problem of whether the agents used provided their protection through action on the breakage mechanism or the recovery mechanism. The work in this laboratory for the past few years has been concerned primarily with breakage and many procedures have been utilized to study effectively this aspect of chromosome aberration production. Since breakage cannot be studied directly, since aberration production is important as the measuring device for radiation damage, and since a two-hit aberration involves both breakage and rejoining, experimental procedures were employed, the results of which it was hoped would contribute to a better understanding of rejoining and indirectly of breakage. In nearly all experiments a total dose of 400r of X-radiation was used. The gas atmosphere in the exposure chamber varied from anoxia (obtained by the use of vacuum or helium) through different percentages of oxygen to three additional atmospheres of pure oxygen.

With irradiation in five percent oxygen, by varying the intensity from 1r to 400r per minute and/or the temperature from 0.3°C to 45°C, an average aberration yield varying from about 0.88 per cell to 0.48 was obtained. Although neither the actual number of chromosome breaks nor the distribution of these breaks within the nucleus can be determined, for the following reason we have concluded that 400r of X-radiation in five percent oxygen always produces a similar number of recoverable breaks and that the variations in aberration yield are due to the rejoining variable. In any experiment in this series having less than 0.88 aberrations per cell, by manipulation of the experimental procedure—using temperature changes or respiratory inhibitors, either with pre- or posttreatment or during irradiation—the breaks in the form of aberrations can be recovered, so this maximum of 0.88 is obtained.

In those experiments where conditions of anoxia (mostly by use of helium) were employed at different intensities and different temperatures, the highest aberration yield obtained was 0.66 per cell, while a low of 0.14 was found. Also in these experiments in helium where an aberration yield below the maximum was found, by certain manipulations in the experimental procedure, the breaks could be recovered in the form of aberrations to give yields up to but not higher than the 0.66 per cell. Again the conclusion was reached that 400r of X-radiation in helium produces a constant number of recoverable breaks and that the different aberration yields recorded are dependent solely upon the rejoining process.

Helium, air or five percent oxygen were used as the gas component of the

exposure chamber. The only agent found to influence the number of chromosome breaks is the oxygen concentration during irradiation. Any means to increase or decrease the oxygen concentration at the time of irradiation will directly influence the number of breaks. In these experiments the oxygen concentration has been varied by the use of different percentages of oxygen in the exposure chamber and by using different temperatures to alter the amount of dissolved oxygen. When oxygen was used in an experiment, those environmental factors which might influence the amount of oxygen in solution during irradiation were standardized so that a constant number of breaks could be anticipated for each experimental design. Since we believe that 400r of X-radiation for any given experimental condition will produce a constant number of recoverable chromosome breaks, the present investigations were concerned with the influence of postirradiative changes on the rejoining process as expressed in aberration yield.

In Table 1 the data from experiments where irradiation took place in an atmosphere of five percent oxygen, with CO used either during or after irradiation, indicate that the CO effect is operative on the rejoining mechanism and demonstrate a postirradiative effect. Since CO inactivates the action of cytochrome oxidase, thus inhibiting the release of energy, it is assumed that the broken ends of the chromosomes are prevented from rejoining through a reduction in the energy necessary for this process. In Table 2 results are given from material irradiated in air followed by posttreatment in either air or helium, while the data recorded in Table 3 were from material irradiated in helium and posttreated either in helium or air. Following irradiation in air with posttreatment in helium, there is an increase in aberration yield, while cells irradiated in helium followed by posttreatment in air show a decrease in aberrations. These data, besides demonstrating a posttreatment effect, also indicate that postirradiative anoxic treatment favors new reunions over restitution—probably by reducing the amount of energy available for rejoining—and that posttreatment in air, because of the rapid supply of energy through oxidative metabolism, favors restitution over new reunions. This influence of oxygen on energy relationships has been considered by SWANSON (1955), WOLFF and LUIPPOLD (1955) and BEATTY and BEATTY (1957).

From the data presented in experiments 3–8 it appears that after ten minutes the posttreatment effect of CO is nonoperative in modifying the aberration yield. This does not mean necessarily that all the breaks have healed or restituted during this time, for as has been calculated by LEA (1947) and WOLFF *et al.* (1958), two breaks have to be about a micron or perhaps much less in distance from each other in order to be effective in forming two-hit aberrations. One reason for using fractionated doses, as was done in most of the work reported here, is that use can be made of those breaks produced in dose I that are too far apart to form exchanges by giving the same material a second dose, thereby making additional breaks available for interaction.

The data in Tables 4 and 5 are the result of certain fractionation procedures—

in Table 4 with air as the gas between the two 200r fractions and in Table 5 with helium. The experimental data in Table 4 indicate when the first dose was given in air and material left in air during various periods between doses, that the two doses cease to be synergistic and become additive at the 30-minute interval (experiment 23). In the same fashion the data indicate, when irradiation took place in helium and the same time intervals between doses were used with air as the intervening gas, that the two fractions also cease to be synergistic and become additive at the 30-minute interval. This would indicate that breaks made either in air or helium remain active the same length of time. WOLFF and LUIPPOLD (1958), using somewhat the same general procedure in studying modification of chromosomal aberrations, used nitrogen instead of helium. They found that "Tradescantia breaks induced in nitrogen close too quickly to allow easy administration of postirradiation treatments".

Since our data indicate that in the procedures used here the breaks made in helium or in air remain active the same length of time and since they indicate that a posttreatment effect can be obtained easily, the experiments from which the data are recorded in Tables 4–7 were carried out to explore the nature of this posttreatment effect. BEATTY and BEATTY (1957) suggested that the systems furnishing energy for rejoining in an oxygen and in an oxygen-free atmosphere differ; that when oxygen is present oxidative metabolic processes are involved, but in anoxic conditions anaerobic metabolism is involved. Since the working hypothesis for these experiments was that the amount of energy available for rejoining directly influenced the aberration yield, two factors directly concerned with the energy relationship were the variables used. These were the type of posttreatment and the temperature.

A comparison of the results of experiments 2 and 3 suggests two things. First, in an aerobic atmosphere there is apparently no reserve supply of energy available for chromosome rejoining which is not influenced by the action of CO. Second, the anaerobic system is not capable of producing at this temperature and duration of time much additional energy over and above the basic requirements of the cell. Since Tradescantia inflorescences were able to live in pure CO, in the dark, for four hours without any noticeable ill effect (BEATTY and BEATTY 1959), it would appear that anaerobic metabolism is utilized by this plant. In Tables 2–6 where results of fractionated doses are reported, in the experiments where air was used following irradiation, rejoining took place, but when helium was used rejoining was inhibited. In the anoxic condition obtained by using helium, it appears that the basic energy requirements of the cell determines how much energy is left over to be used by the chromosomes for rejoining. BEATTY and BEATTY (1959) found when helium was used throughout the experiment and only the temperature varied, that at 0.3°C the aberration yield was 0.14; at 30°C it was 0.26; and at 40°C it was 0.66. The explanation suggested for these results was that at the low temperature only a small amount of energy was necessary for maintenance, so some energy was available to be used by the chromo-

somes for rejoining and at 40°C all energy was needed by the cell for basic requirements, leaving none for rejoining. The experimental data in Table 3 lend support to this conclusion, for when air was used immediately after irradiation in helium, the aberration yield decreased from 0.20 to 0.11 for a single dose of 200r and from 0.66 to 0.26 for a 400r dose, indicating that when energy was made available the chromosomes had more energy for rejoining, which favored restitution over new reunions. On the other hand considering the results given in Table 2, where irradiation took place in air followed by posttreatment in helium, one can assume that the available energy was decreased, thus ultimately favoring new reunions over restitution. The results reported by BEATTY and BEATTY (1959) where one temperature, 30°C, was used with helium but the amount of energy available was varied by using anaerobic respiratory inhibitors, indicated that a decrease in available energy increased the aberration yield. In some current work in this laboratory, in a helium atmosphere throughout the experiment, at 30°C, a posttreatment with some chemical compounds which are believed to be involved in metabolism has decreased the aberration yield.

In the experiments reported in Table 7, irradiation was done in air at a low temperature, since previous results (BEATTY and BEATTY 1959) indicate that the enzymes involved in oxidative metabolism were inhibited at 0.3°C. The results in Table 7 show that this temperature, under these experimental conditions, also is effective in increasing aberration yield compared to results in Tables 4 and 5 at 30°C. The results indicate that when air is used as the intervening gas between doses of X-radiation, some rejoining took place during the 30-minute rest period, so that apparently the inactivation of the enzymes under these conditions is not complete. With helium between doses the aberration yield is maintained for this period of time, due perhaps both to low temperature and insufficient oxygen.

All the experimental results reported here, as well as those already reported in other publications in this series of investigations, indicate that the rejoining of broken chromosomes is dependent upon energy relationships in the cell, including both the anaerobic and the aerobic respiratory systems. Irradiation does not seem to affect the operation of the energy-supplying systems, for if conditions are favorable the systems seem capable of supplying energy even during irradiation as well as immediately after. The experimental results seem to indicate that there is competition for energy in the cell and that some of the basic processes in the cell have priority over the demand for repairing broken chromosomes. Evidently, through the evolutionary process in the development of the species, the supply and demand for energy in the cell has been fairly well balanced. The breaking of chromosomes and the need for additional energy for repair has imposed on the cell new requirements. The cell under normal conditions apparently does not have much energy stored, nor the mechanism for immediate increase in energy production. It might be also that there is no well-established pathway of energy transfer to broken chromosomes, since chromosome repair of the magnitude induced by radiation is foreign to the cell. It is only when by different experimental

procedures the energy requirements for the normal processes in the cell can be reduced or when external factors can be utilized to increase energy production in the cell that additional energy is available for chromosome repair, which subsequently lessens the permanent damage. The reverse seems also to be true—that increasing the basic energy requirements of the cell reduces the amount of energy available for chromosome repair.

## SUMMARY

The CO effect in relation to chromosomal aberration production in oxygen is operative on the recovery mechanism since immediate postirradiative treatment with CO yielded the same number of aberrations as irradiative treatment. As determined by the CO effect, all rejoining, as far as two-hit aberrations are concerned, was completed within ten minutes after irradiation. All experiments in which irradiation was done in air followed by an immediate postirraditive treatment in helium, showed a posttreatment effect by an aberration yield higher than was obtained with posttreatment in air. In all the experiments where helium was used followed by air a decrease in aberration yield was found. These data are interpreted as supportive evidence that the availability of energy for chromosome rejoining directly influences the two-hit aberration yield in such a way that postirradiative aerobic conditions favor restitution over exchanges while postirradiative anoxic conditions favor exchanges over restitution. The results from fractionation experiments indicate that some of the breaks produced in air or in helium when postirradiatively treated in air remain open and available for use in the production of two-hit aberrations for approximately 30 minutes, and that breaks postirradiatively treated in helium remain open for a longer period.

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# SOMATIC CROSSING OVER AND ITS GENETIC CONTROL IN DROSOPHILA

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IN Drosophila, somatic crossing over in the hypodermal cells provides a means of exploring developmental patterns and of studying the effects of certain genes in specialized groups of cells. Mitotic recombination makes certain genes homozygous in parts of the diploid organism. The fact that crossing over can take place in somatic cells as well as in the germ line has led to the suggestion that the genetic analysis of slow-breeding animals such as man might be accomplished in tissue culture. Somatic mosaicism resulting from somatic recombination is a relatively rare phenomenon, however, even in Drosophila, and has not been detected in other higher animals. It might be supposed a selective advantage would be conferred by factors preventing this type of exchange, with its potential for uncovering lethal or deleterious genes.

Mitotic crossing over has been demonstrated in several species of filamentous fungi (PONTECORVO 1956) and in two species of yeast (ROMAN 1956; LEUPOLD 1958). PONTECORVO has emphasized its importance as one of the means, along with transduction, transformation and lysogenesis, for conferring genetic plasticity on an organism which lacks a sexual phase in its life cycle. These parasexual means of recombination make possible the genetic analysis of asexual microorganisms, and give them a means of reassorting genes, thus conferring a potential for evolutionary adaptability.

BRIDGES (1925) was the first to investigate somatic mosaicism in Drosophila. His interpretation was that an X chromosome carrying a Minute was occasionally eliminated, thus revealing the phenotype of recessive mutants on the homologous chromosome. STERN (1927) obtained similar data for the autosomes which apparently corroborated BRIDGES' hypothesis. Later, however, STERN (1936) was able to establish that the mechanism producing the mosaic effects was due to somatic crossing over which took place at the four-strand stage. The centromeres disjoin, so that half of the segregations produce cells homozygous for loci originally heterozygous. If linked recessive mutants are on opposite members of a homologous pair of chromosomes and can produce a phenotype visible in the hypoderm (such as yellow and singed on the X chromosomes which can be identified in single bristles), then a crossover between the centromere and the proximal locus can lead to a twin spot, which consists of two adjacent patches of mutant tissue on a wild type background; if the cell lineage of one of the crossover products does not contribute to hypoderm in which the

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mutant phenotype is detectable, then only a simple yellow or singed spot results. A crossover between the two markers can lead to tissue homozygous for the distal locus, yellow, but not for singed. Double crossovers are thought to be rare (STERN 1936; KAPLAN 1953).

Several studies have been concerned with the effect of nongenetic variables on somatic crossing over in Drosophila. Various workers have used temperature (STERN and RENTSCHLER 1936; KAPLAN 1953; BROSSEAU 1957), ionizing radiation (LEFEVRE 1948; BECKER 1956) and maternal aging (SCHWARTZ 1954; BROWN and WELSHONS 1955).

Although there is no reason to postulate a fundamental difference in mechanism between meiotic and mitotic crossing over (PONTECORVO 1958), they may differ greatly in their response to modifying genetic factors. The agents which prevent meiotic crossing over in Drosophila males do not affect somatic crossing over, which occurs in both sexes (STERN 1936; KAPLAN 1953). The *C3G* gene does not alter the frequency of mosaicism due to mitotic exchange, although it almost totally prevents meiotic crossing over in Drosophila females (LE CLERC 1946).

The group of genetic factors known as the Minutes exert a profound effect upon the incidence of somatic crossing over in Drosophila. The presence of a Minute, especially in the same chromosome arm as that in which the crossovers are being measured, increased the frequency of mitotic recombination (STERN 1936; KAPLAN 1953). Minutes occur throughout the chromosome complement, and all produce a similar phenotype chiefly characterized by prolonged larval development, slender bristles, and lowered viability and fertility. They act as dominants, are generally thought to be small deletions, and are lethal when homozygous.

The existence of other genetic factors affecting somatic crossing was indicated by BROWN and WELSHONS (1955) who found great variation in the incidence of mosaicism among the several stocks which they employed. They supposed that this was due to differences in autosomal constitution of the stocks, since the X chromosomes were the same in each experiment.

Relative frequencies of meiotic and somatic crossing over are quite different for specific chromosome regions. STERNS's experiments using the Theta translocation (which is a fragment of the X attached to the right arm of an intact X chromosome) indicated that somatic crossing over took place chiefly in the proximal heterochromatin. KAPLAN (1953) using the second chromosome marker Bristle (*Bl*) found that more somatic crossing over took place in the centromere-*Bl* region, 0.2 map units long, than in the distal *Bl-S* region, 41.9 units long. BROWN (personal communication) found that somatic crossing over was very high in a special X chromosome which contained extra heterochromatin, and very low in an X which lacked the normal amount of heterochromatin. In contrast, meiotic crossing over is almost totally absent in centromeric heterochromatin. The relative lengths of intervals in *Aspergillus nidulans* mapped by means of mitotic crossovers are different from those mapped meiotically (PONTECORVO and KÄFER 1956).

In view of the increased use of mitotic recombination as a tool for genetic and developmental analysis and of its apparent correspondence in basic mechanism to meiotic crossing over, it seemed worthwhile to initiate a study of the genetic control of somatic crossing over. Drosophila was chosen because of the available knowledge and techniques for analyzing mosaics. The experiments reported below were designed to detect and characterize genetic factors controlling somatic crossing over in Drosophila.

## MATERIALS AND METHODS

Two unrelated lines were synthesized, both carrying the sex-linked markers *y* and *sn*$^3$, in addition to other recessive markers on all the major chromosomes. It was hoped that there would be some consistent difference in X chromosome somatic crossing over characteristics between these two lines, and that genetic factors responsible for the difference could be located.

Crossovers between the marked X and the X from the balancer stock were utilized to recover two stocks from each line, one homozygous *y* +, the other + *sn*$^3$. The four stocks used in the following experiments consisted of the following genotypes, all mutant loci being homozygous. (Symbols used to identify mutants used in the paper are as follows: *al*–aristaless, *bw*–brown, *cn*–cinnabar, *h*–hairy, *ri*–radius incompletus, *ro*–rough, *sd*–scalloped, *sn*$^3$–singed$^3$, *y*–yellow.)

1. *y sd, al, h ro* } derived from line I, isogenic for chromosomes 2 and 3
2. *sn*$^3$ *sd, al, h ro* }
3. *y, cn bw, ri* } derived from line II, isogenic for chromosomes 2 and 3
4. *sn*$^3$, *cn bw, ri* }

These stocks were maintained by single pair matings to increase homozygosity for the X and fourth chromosomes ,and to maintain isogenicity. Lowered fertility in Stocks 1 and 2 made occasional mass matings necessary. The flies were raised on standard cornmeal-molasses-agar medium at 25° ± 1°C. A vitamin solution added to the medium was found useful in increasing the viability and fertility of Stocks 1 and 2 and had no demonstrable effect on the incidence of somatic crossing over.

A check of the salivary chromosomes of each stock, singly and in several combinations, revealed no recognizable chromosomal aberrations.

For the determination of somatic crossover frequency in each of the unrelated lines, reciprocal crosses were made between Stocks 1 and 2, and between 3 and 4. Five pairs of flies were used in each bottle, and different bottles were considered to be separate cultures although they might be duplicate crosses. The female $F_1$ offspring were scored for incidence of mosaicism by techniques outlined below. These results are presented in Table 1.

For the experiments using flies which bore different combinations of whole autosomes derived from the two parental lines, it was necessary to make up crosses according to the following scheme. Reciprocal crosses were made between the two singed stocks (2 and 4). The male progeny of these two crosses were of two types depending upon the source of the X chromosomes. Each of these two

TABLE 1

*Somatic crossing over in female progeny of crosses within lines*

| Cross (♀×♂) | No. of abdomens | No. of abdomens with spots | Mutant spots | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | Total | |
| | | | Yellow | Singed | Twin | No. | Frequency (spots/abdomen) |
| Low line* | | | | | | | |
| (a) $y \times sn^3$ | 177 | 101 | 55 | 59 | 40 | 154 | 0.87 |
| (b) $y \times sn^3$ | 20 | 8 | 4 | 5 | 3 | 12 | 0.60 |
| (c) $y \times sn^3$ | 74 | 39 | 28 | 13 | 15 | 56 | 0.76 |
| (d) $y \times sn^3$ | 50 | 30 | 19 | 12 | 11 | 42 | 0.84 |
| (e) $sn^3 \times y$ | 20 | 14 | 9 | 7 | 4 | 20 | 1.00 |
| (f) $sn^3 \times y$ | 112 | 59 | 35 | 19 | 22 | 76 | 0.68 |
| Totals for low line | 453 | 251 | 150 | 115 | 95 | 360 | 0.80 |
| Heterogeneity P | | | 0.66 | 0.08 | 0.99 | 0.37 | |
| High line† | | | | | | | |
| (a) $y \times sn^3$ | 64 | 52 | 37 | 35 | 30 | 102 | 1.59 |
| (b) $y \times sn^3$ | 70 | 52 | 41 | 41 | 39 | 121 | 1.73 |
| (c) $y \times sn^3$ | 30 | 21 | 12 | 16 | 13 | 41 | 1.37 |
| (d) $y \times sn^3$ | 32 | 27 | 20 | 23 | 19 | 62 | 1.94 |
| (e) $sn^3 \times y$ | 58 | 47 | 39 | 45 | 26 | 110 | 1.90 |
| (f) $sn^3 \times y$ | 94 | 71 | 32 | 53 | 68 | 153 | 1.63 |
| (g) $sn^3 \times y$ | 40 | 34 | 18 | 23 | 19 | 60 | 1.50 |
| (h) $sn^3 \times y$ | 50 | 44 | 31 | 25 | 38 | 94 | 1.88 |
| Totals for high line | 438 | 348 | 230 | 261 | 252 | 743 | 1.70 |
| Heterogeneity P | | | 0.07 | 0.62 | 0.13 | 0.44 | |

* Genotype of flies scored: $\frac{y + sd}{+ sn^3 sd}, \frac{al}{al}, \frac{h\ ro}{h\ ro}$

† Genotype of flies scored: $\frac{y +}{+ sn^3}, \frac{cn\ bw}{cn\ bw}, \frac{ri}{ri}$

$F_1$ classes was crossed to females of each yellow stock (1 and 3). There were four crosses, and the female offspring of each cross comprised four classes as a result of segregation of the two major autosomes. Each parental chromosome was intact since the heterozygous parent was male. The recessive markers of both stocks made it possible to identify each chromosome combination without ambiguity. All 16 classes were scored for incidence of somatic mosaicism. These results are summarized in Table 2.

A second set of crosses similar in some respects to those outlined above was made in an effort to determine whether the marker genes themselves might be responsible for the differential effects of whole chromosomes on crossing over. The difference between them lay in the fact that $F_1$ heterozygous females were used for crossing to the two types of yellow males. Progeny were obtained which were homozygous only for single marker genes, owing to crossing over in the female parent. The flies which were selected for study of mosaicism were those

which were homozygous for a single mutant locus and heterozygous for all the others. These results are presented in Table 5.

Flies to be examined for mutant patches were collected daily, the males discarded, the females aged and frozen, and the abdomens mounted on microscope slides and examined according to standard techniques (BROWN and WELSHONS 1955). Slides were coded by another person, to preclude subjective bias in the scoring of mutant bristles.

Yellow and singed bristles could almost invariably be unequivocally identified. Certain errors could result, but it is likely that those factors which tend to prevent accurate identification of bristle phenotype are either small or tend to balance each other. In the present study the numbers of yellow and singed bristles constituting twin spots were almost equal.

It often happened that mutant bristles were interspersed with wild type ones. It was occasionally a matter of some doubt whether a group of several mutant bristles resulted from one or from more than one crossover event. The fact that each tergite is formed by the growth of cells from the outer margin of the abdomen towards the center (BODENSTEIN 1950) restricted any spot to a half tergite. It also formed a basis for a reasonable judgment about the pattern to be followed by a group of cells with a common descent. However, in doubtful cases, an arbitary standard was adopted of classifying mutant bristles separated by more than one fourth tergite of wild type setae as two independent spots, and those separated by less than one fourth tergite of wild type setae as a single spot.

## RESULTS

### *Characteristics of somatic crossing over within lines*

The questions to be considered in this section concern the general characteristics of somatic crossing over in the stocks selected for these experiments. The types and numbers of mutant spots on the abdomens of the female offspring of the crosses within the two unrelated lines are given in Table 1. These were analyzed statistically for the purpose of determining whether there were differences in frequency distribution and size.

*Frequency of crossing over:* There was an average of 0.80 spots per abdomen in the line bearing *sd*, *al*, *h ro* and 1.70 spots per abdomen in the other line, a highly significant difference ($P<0.001$). If the spots of each phenotype are considered separately, the differences are still very significant. As a result of these findings, the *cn bw*, *ri* line was designated the high line (H), and the *sd*, *al*, *h ro* line was designated low line (L).

*Reproducibility of crossover frequency:* Numbers of yellow, singed and twin spots are recorded for each culture in Table 1. A $\chi^2$ test revealed that there were no significant deviations from the means within each line in spite of the fact that the last cultures were raised more than a year after the first.

*Independence of crossovers:* The probability that any given bristle on a fly abdomen will be mutant is extremely small; there are almost 700 bristles per abdomen, but the maximum number of mutant bristles observed on a single

abdomen was 24, and the maximum number of separate spots only eight. The positional distribution of the nonwild type setae is, to a large extent, random. Therefore, each individual may be considered a sample, and the number of abdomens possessing zero, one, two and more spots should follow a Poisson distribution, providing the events leading to the formation of a spot are all random. A chi-square comparison of the observed distribution agreed with that expected in a Poisson for both L ($P = 0.98$) and H ($P = 0.41$).

*Spot frequency and spot phenotype:* BROSSEAU (1957) found that spot frequency could be considered independently of spot phenotype. This finding implies that every mutant patch arises from the same type of exchange, namely a crossover between the locus of singed and the centromere. The occurrence of nontwin spots is explained by the assumption that the reciprocal product of segregation simply did not happen to form tissue with bristles. Thus a nontwin or simple spot may be considered a "potential" twin if the somatic exchange leading to its occurrence took place between the proximal marker and the centromere. Other possible causes of somatic mosaicism, such as somatic mutation, chromosome elimination or heterochromatic position effects are considered to be negligible. If these assumptions are valid, then the ratio of yellow to singed spots should not depart significantly from unity. The observed numbers of spots of these phenotypes are, in general, equal. The only exception is that of the low-line data (Table 1), where a ratio of 150 yellow to 115 singed spots ($P = 0.03$), is due mainly to cultures (c) and (f). It was felt that this was merely an unusually large random deviation. This conclusion was supported by the following consideration. A count of all abdomens having two simple spots (i.e., nontwin) would be expected to yield a ratio of one quarter with two yellow spots, one half with one yellow and one singed spot, and one quarter with two singed spots. Data from such abdomens agree closely with the expected ratio ($P = 0.95$). It seemed reasonable, therefore, to consider total mosaicism in making statistical comparisons.

*Distribution of spots on the abdomen:* STERN (1936) found that there were preferred regions on the abdomen for the occurrence of each type of spot; BROSSEAU (1957), on the other hand, found that the incidence of spotting on any given tergite was proportional to the number of bristles on that segment. The data presented in Table 1 were obtained by methods essentially identical with BROSSEAU's, and yet it was found that there were far fewer mutant patches than were expected on the posterior tergites according to the hypothesis that the probability of spotting is proportional to the number of bristles. Assuming independent Poisson distribution on each tergite, there is a significant anterior-posterior gradient for the occurrence of mitotic crossing over in tergites 4, 5 and 6; there is no indication of a gradient, however in tergites 2, 3 and 4.

*Size of mutant spots:* It is possible for two stocks to differ in the time at which crossing over takes place. Crossovers occurring early in the individual's development would be expected to lead to mutant spots larger than those arising from crossovers occurring later. All the spots in each stock were classified as to bristle

number, and a mean spot size was computed. There is no evidence of differences between high or low lines in the time of crossing over: the mean spot sizes are quite similar. There are no simple patches larger than seven bristles in the 453 abdomens of line L, and only one as large as nine bristles in line H. The largest twin spots had 16 and 14 bristles respectively. Whatever factors induce somatic crossing over in the abdomen evidently are active only rather late in development.

### *Somatic crossing over in the female offspring of crosses between high and low lines*

The two lines discussed in the previous section differed sharply in their frequencies of somatic crossing over. The factors distinguishing these lines can be expected to segregate in an $F_2$ or backcross, providing crossing over is dependent on the chromosomal constitution. Results presented in this section show that the frequency of somatic crossing over depends on factors borne by all three major chromosomes.

In this set of four crosses the male parents were heterozygotes between the two singed stocks, and the female parents were homozygous yellow flies of Stocks 1 and 3. Sixteen classes of recombinants were obtained which bore various combinations of intact chromosomes from the high and low lines. The numbers of mutant spots in each of these classes are recorded in Table 2. Each chromosome is identified with an H or an L, depending on whether it came from the high or the low line. Each class was made up of flies coming from at least three subcultures. The total female progeny of a cross was used unless it was unusually fertile, in which case only an unselected portion was mounted and mapped.

Chi-square calculations were done on the numbers of yellow, singed, and twin spots, and the totals of all three, in order to test the homogeneity between cultures within each of the 16 classes. No P was less than 0.05, indicating that the frequency of somatic crossing over for each of the genotypes is a stable and reproducible characteristic. The number of abdomens having 0, 1, 2, 3 or more spots was tested for its conformance with the Poisson distribution for each of the 16 classes, using chi-square in the same manner as for the L × L and H × H crosses. No significant deviation from a random distribution of spots was found.

A contingency test was made upon the expectation that the proportions of yellow, singed and twin spots remained the same in all 16 groups, despite the fact that the total amount of mosaicism varied considerably. These proportions are remarkably similar ($P>0.7$).

The implications of the data in Table 2 may now be considered. Class 1 has the same genetic content as does the low line, and spot frequencies of the two groups are rather close: 0.72 spots per abdomen for the former, 0.80 for the latter ($P = 0.37$). At the end of the table, class 16 has a spot frequency of 1.72 spots per abdomen, which checks well with the high line value of 1.70 ($P = 0.9$). Thus it is possible, by bringing together the chromosomes originally present in the parents, to reassemble the factors responsible for the characteristic high and low rates of somatic crossing over.

TABLE 2

*Incidence of somatic mosaicism in flies resulting from crosses between lines*

| Cross | Class | Genotype* 1 | 2 | 3 | No. of sub-cultures | No. of flies | Yellow spots no. | Singed spots no. | Twin spots no. | Totals No. | P† | Frequency | $\frac{s}{\bar{x}}$‡ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Stock 1 × | (1) | L/L | L/L | L/L | 10 | 114 | 26 | 35 | 21 | 82 | 0.99 | 0.72 | 0.079 |
| L, L/H, L/H | (2) | L/L | L/L | L/H | 3 | 99 | 30 | 25 | 17 | 72 | 0.35 | 0.73 | 0.086 |
| (singed) | (3) | L/L | L/H | L/L | 4 | 101 | 37 | 30 | 25 | 92 | 0.71 | 0.91 | 0.095 |
| | (4) | L/L | L/H | L/H | 3 | 100 | 41 | 36 | 28 | 105 | 0.47 | 1.05 | 0.102 |
| Stock 1 × | (5) | L/H | L/L | L/L | 10 | 105 | 22 | 18 | 24 | 64 | 0.16 | 0.61 | 0.076 |
| H, L/H, L/H | (6) | L/H | L/L | L/H | 4 | 120 | 28 | 23 | 23 | 74 | 0.08 | 0.62 | 0.072 |
| (singed) | (7) | L/H | L/H | L/L | 4 | 112 | 32 | 38 | 20 | 90 | 0.92 | 0.80 | 0.085 |
| | (8) | L/H | L/H | L/H | 3 | 100 | 42 | 27 | 29 | 98 | 0.39 | 0.98 | 0.099 |
| Stock 2 × | (9) | L/H | L/H | L/H | 3 | 102 | 44 | 39 | 26 | 109 | 0.75 | 1.07 | 0.102 |
| L, L/H, L/H | (10) | L/H | L/H | H/H | 3 | 102 | 46 | 36 | 31 | 113 | 0.13 | 1.11 | 0.104 |
| (singed) | (11) | L/H | H/H | L/H | 3 | 101 | 49 | 43 | 40 | 132 | 0.98 | 1.31 | 0.114 |
| | (12) | L/H | H/H | H/H | 3 | 101 | 60 | 51 | 50 | 161 | 0.60 | 1.59 | 0.126 |
| Stock 3 × | (13) | H/H | L/H | L/H | 3 | 118 | 61 | 56 | 36 | 153 | 0.82 | 1.30 | 0.105 |
| H, L/H, L/H | (14) | H/H | L/H | H/H | 3 | 112 | 53 | 56 | 53 | 162 | 0.94 | 1.45 | 0.114 |
| (singed) | (15) | H/H | H/H | L/H | 3 | 119 | 52 | 54 | 53 | 159 | 0.05 | 1.34 | 0.106 |
| | (16) | H/H | H/H | H/H | 3 | 123 | 78 | 68 | 66 | 212 | 0.99 | 1.72 | 0.118 |
| Totals | | | | | 65 | 1729 | 701 | 635 | 542 | 1878 | | | |

* L refers to a chromosome derived from the low line; H to one derived from the high line (see RESULTS for gene markers).
† Based on $\chi^2$ test for heterogeneity in the subcultures.
‡ Standard deviation of the mean.

Classes 8 and 9 consist of segregants that are heterozygous for all three major chromosomes, and are genetically identical with $F_1$ heterozygotes between high and low lines. Four additional crosses were made leading to the same genetic makeup as is present in classes 8 and 9 (Stocks 1 × 4, 4 × 1, 3 × 2 and 2 × 3). Table 3 gives the results from all six of these crosses. There are no significant deviations from the mean among them.

The remaining 12 classes of Table 2 have various combinations of H and L chromosomes, and the effect of substitution of an H chromosome for an L chromosome may be calculated. To accomplish this, pairs of classes were compared which differed only in one chromosome; the other five chromosomes being identical. Four such pairs existed for each of the six possible chromosome substitutions, and a constant was derived from each set of comparisons, which represented the increase in average frequency that could be expected from each substitution of H for L. In order for these constants to reflect properly the various sizes of each class, they were weighted by the reciprocals of the error variances. Table 4 summarizes these determinations. Chi-squares were done for each of the constants to determine whether the differing chromosomal content of each group was affecting the action of the measured chromosome. These were low enough to indicate that there was no significant interaction between chromosomes.

TABLE 3

*Incidence of mosaicism in $F_1$ flies resulting from crosses between high and low lines*

| Class | Total no. abdomens | Yellow | Singed | Twin | Totals | Frequency |
|---|---|---|---|---|---|---|
| (8)* L/H, L/H, L/H | 100 | 42 | 27 | 29 | 98 | 0.98 |
| (9)* L/H, L/H, L/H | 102 | 44 | 39 | 26 | 109 | 1.07 |
| Cross-(1) Low yellow × high singed | 108 | 40 | 35 | 31 | 106 | 0.98 |
| Cross-(2) High singed × low yellow | 115 | 38 | 25 | 27 | 90 | 0.78 |
| Cross-(3) High yellow × low singed | 130 | 39 | 36 | 36 | 111 | 0.85 |
| Cross-(4) Low singed × high yellow | 103 | 38 | 44 | 29 | 111 | 1.08 |
| Totals | 658 | 241 | 206 | 178 | 625 | 0.95 |
| Heterogeneity $\chi^2$ | | 3.937 | 10.388 | 0.950 | 8.144 | |
| Heterogeneity P | | 0.56 | 0.07 | 0.95 | 0.15 | |

* See Table 2.

TABLE 4

*Summary of contribution each H chromosome to the incidence of mosaicism*

| Chromosome | Substitution | Weighted mean freq. increase ($D_i$) (spots/fly) | Variance ($s^2$) | $s_{\bar{x}}$ (standard dev. of the mean) | Background P |
|---|---|---|---|---|---|
| X | L/H for L/L | −0.102 | .0037 | 0.060 | 0.99 |
| | H/H for L/H | 0.186 | .0061 | 0.078 | 0.55 |
| 2 | L/H for L/L | 0.263 | .0038 | 0.061 | 0.67 |
| | H/H for L/H | 0.249 | .0062 | 0.078 | 0.25 |
| 3 | L/H for L/L | 0.069 | .0037 | 0.060 | 0.68 |
| | H/H for L/H | 0.205 | .0061 | 0.078 | 0.40 |

For identification of genes in H and L, see Table 1.

A constant, $A$, representing an estimate of the base frequency on which the added factors were operating was derived from the expression

$$A = \frac{\Sigma\,(O_i W_i) \;-\; \Sigma\,(D_i W_i)}{\Sigma W_i}$$

where $O_i$ = observed frequency (spots/abdomen) for each class.

$D_i$ = constant or sum of constants (see Table 4) expected for each class.

$W_i$ = weighting factor [(no. of abdomens)$^2$/ no. of spots].

Since $A$ may be considered to correspond to the somatic crossover frequency of the L line, the value for this constant ought to check fairly well with the direct measurement on that line. The value of $A$ is determined to be 0.73. This compares very favorably with the L/L, L/L, L/L value from Table 2, which is 0.72 spots/abdomen.

One can now test the observed frequencies of each class in Table 2 against the expected frequencies obtained as the sum of $A$ and the added constants appropriate for each group. This determination indicates whether the calculated chromosome contributions are adequate to explain the observed size of each class; a high

$x^2$ would indicate either nonadditive interactions between chromosomes or uncontrolled factors, possibly nongenetic in nature. The $x^2$ is 12.83 (9 d.f., P = 0.17) using the values $A = 0.73$ and the six constants listed in Table 4. It would seem that differences in frequency of somatic crossing over between the genotypes tested can be considered to be controlled completely by factors on the three major chromosomes.

Since the two stocks in these experiments differ in the markers they carry, the question arises as to whether the mutant loci themselves exert this control. Backcrosses were made which were similar to the ones used for Table 2, except that the heterozygous $F_1$ parents were female. Backcross progeny were recovered which were homozygous for one mutant locus only, and the heterozygous for all other recessive markers. If any one of these marked regions was exerting an effect on somatic crossing over, differences between these classes of flies should reveal it. Results are given in Table 5. There is a surprising similarity in frequencies of somatic crossing over for each different phenotype. Indeed, a heterogeneity $x^2$ comparison indicates that there is no significant variation from the mean among them, even if all classes with high markers are compared with all classes with low markers. It can safely be asserted then, that it is not the visible mutants which are controlling the frequencies of mitotic exchange, nor are the controlling factors closely linked with any of the recessive markers.

TABLE 5

*Somatic crossing over in backcross progeny homozygous for single mutant loci*

| | Mutant phenotype | No. of abdomens | Yellow spots | Singed spots | Twin spots | Total spots | Frequency |
|---|---|---|---|---|---|---|---|
| Low line markers | *sd* | 122 | 34 | 46 | 43 | 123 | 1.01 |
| | *al* | 82 | 15 | 25 | 25 | 65 | 0.79 |
| | *h* | 69 | 17 | 20 | 22 | 59 | 0.86 |
| | *ro* | 80 | 23 | 23 | 21 | 67 | 0.84 |
| High line markers | *cn* | 98 | 28 | 35 | 21 | 84 | 0.86 |
| | *bw* | 102 | 26 | 21 | 31 | 78 | 0.77 |
| | *ri* | 118 | 46 | 36 | 29 | 111 | 0.94 |
| Totals | | 671 | 189 | 206 | 192 | 587 | 0.88 |
| Heterogeneity P | | | 0.23 | 0.25 | 0.56 | 0.50 | |

## DISCUSSION

The experiments described herein lead to the conclusion that there are chromosomal factors other than Minutes which are not associated with any visible deficiency or aberration but which control the frequency of somatic crossing over. These are located both on the X chromosome where crossing over is being measured and also on chromosomes 2 and 3; the latter thus exert an effect on exchanges in non-homologous chromosomes.

The types of substitutions allow some general observations to be made on the characteristics of the gene or genes in each chromosome. When the X is heterozygous for H/L, there is a lower frequency of crossing over than when either chromosome is homozygous. There are at least three explanations which could be advanced to account for this effect. First, the H chromosome (with high-line markers) could contain a dominant repressor of crossing over, whose effect was relatively slight, producing an average of 0.102 fewer spots on each fly carrying it. There would also need to be postulated a more potent X-linked promoter of crossing over which was primarily recessive, but whose effect when homozygous more than offset the repressor action, so that the net increase of H/H over L/L was $0.186 - 0.102 = 0.084$. If there are such factors, they should be separable by crossing over.

A second possible explanation is suggested by the results of STADLER (1956) in connection with variability of meiotic crossing over in Neurospora. He found that successive generations of inbreeding increased the percent recombination in a marked interval near the centromere, and suggests that small aberrations might limit the closeness of pairing. Since somatic crossing over in Drosophila occurs primarily in the heterochromatin (STERN 1936; KAPLAN 1953) rather large aberrations would not be noticed in the salivary preparations, owing to the diffuseness of the chromocenter.

A third hypothesis that might account for the heterozygous effect would invoke epistatic interaction of gene *A* on L with gene *B* on H in such a way as to suppress somatic crossing over.

The second chromosome of line H contains at least one factor whose action is dominant and apparently additive. One H (H/L) raises the frequency of crossing over by 0.263; another second chromosome H adds 0.249 to the total.

The third chromosome genes that promote somatic crossing over in line H seem to be almost wholly recessive. The classes heterozygous for the third chromosome (H/L) show almost exactly the same frequencies of crossing over as do the homozygous L/L classes, having an average of only 0.069 more spots per fly; yet flies homozygous H/H for chromosome 3 have an increase of 0.205 spots per fly over the heterozygote classes.

An examination of Table 2 reveals that every class having an added H chromosome in both the second and third chromosomes (classes 4, 8, 12 and 16) has a higher frequency of somatic crossing over than would be predicted from purely additive effects of the H-2 and H-3 acting separately. This is not statistically significant, however, as shown by $\chi^2$ tests on the background effect.

The calculated effect of each of the six substitutions summarized in Table 4 can completely account for the observed difference between the original high and low lines. The regularity of the results also demonstrates that somatic crossing over is a precisely controlled process. The genes responsible may affect either the closeness of somatic pairing, or the physical or chemical processes responsible for the exchange of chromosomal segments, or both. Mitotic pairing is not a sufficient condition for mitotic exchange, but it is certainly a necessary one.

The mode of action of Minutes is still not understood. It is possibly due to the longer larval life undergone by Minute individuals. BROSSEAU (1957) has shown that temperature shocks administered during the prepupal stages can affect the incidence of mosaicism in adults. It has been suggested (PERKINS, personal communication) that flies homozygous for the gene *gt* (giant) might be used as a means of prolonging the larval period. The *gt* gene is incompletely penetrant, so that some *gt/gt* flies in a culture may be phenotypically normal while others may go through an extra instar and be considerably larger than wild type adults. The proportion of the two phenotypes is governed by the level of nutrition afforded the larvae. Thus isogenic cultures may be used wherein individuals nevertheless differ sharply in length of larval life.

It has been shown (LEVINE and LEVINE 1955; LAWRENCE 1958) that there are autosomal genes which exert an effect on meiotic crossing over in the X chromosome. The present work demonstrates the existence of a series of genes affecting somatic crossing over in the X. It would be of considerable interest to determine whether the well-known effect of heterozygous inversions on crossing over in non-homologous chromosomes (SCHULTZ and REDFIELD 1951) also has its parallel in mitotic crossing over.

## SUMMARY

Two unrelated lines of *Drosophila melanogaster* were selected without any prior knowledge of their tendencies towards mosaicism. Somatic crossing over was measured in the X chromosome using the classic *y* and *sn*$^3$ system. The two lines differed markedly in their incidence of mosaicism, averaging 0.8 spots per fly, and 1.7 spots per fly, respectively. There was no difference between the two in mean spot size or in distribution of mutant patches.

The frequency of spots resulting from backcrossing $F_1$'s of crosses between the high and low lines revealed that factors governing the frequency of X chromosome somatic crossing over occurred on all three major chromosomes. The recombination classes having the same genetic content as the parents duplicated the parental frequencies of spotting. A high degree of genetic and environmental control was achieved. In experiments with intact (noncrossover) chromosomes, no variation was observed that could not be explained by defined genotypic differences and by expected sampling deviations from a Poisson distribution.

Constants for the contribution of each chromosome to the total mosaicism of the H stock were determined. The values derived from these constants for each segregating class agreed well with the experimental results.

It is concluded that somatic crossing over is a process under the precise control of genetic factors governing the frequency of its occurrence.

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